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EFFECT OF YOGA ON GUT MICROBIOTA MODULATED METABOLISM IN OBESE INDIVIDUALS

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1. Abstract

Yoga, a holistic mind–body practice with millennia-old roots, has demonstrated benefits for stress reduction and metabolic health, yet its influence on the gut microbiome and related metabolic pathways remains underexplored. This dissertation investigates whether a structured 10-week yoga intervention can modulate gut microbial composition and diversity in obese adults and assesses consequential effects on anthropometric, biochemical, and metabolic markers. In a controlled design, 16 participants underwent thrice-weekly yoga sessions, while a matched control group (n=7) maintained usual activity. Outcomes measured pre- and post-intervention included body weight, BMI, waist circumference, lipid profile, insulin levels, oxidative stress markers (malondialdehyde, GSSG), antioxidant capacity (GSH, vitamin C), Insulin and 16S rRNA gene sequencing of faecal samples. The yoga group exhibited significant reductions in weight (–3.2%, $p<0.01$), BMI (–2.8%, $p<0.01$), and waist circumference (–4.5%, $p<0.001$), alongside decreased oxidative damage (MDA –18%, $p<0.01$; GSSG –34%, $p<0.001$) and enhanced antioxidant defences (GSH +31%, $p=0.03$; vitamin C +17%, $p=0.03$) and improved insulin sensitivity (–12%, $p<0.05$). Microbiome analyses revealed increased α-diversity (Observed OTUs +12%, $p<0.05$), significant shifts in β-diversity (Bray–Curtis, PERMANOVA $p=0.02$), normalization of *Prevotella*/*Bacteroides* ratio, and enrichment of short-chain fatty acid–producing taxa (e.g., *Faecalibacterium*, *Blautia*). No comparable changes occurred in the control cohort. These findings establish yoga as an effective adjunctive intervention for obesity, operating through gut microbiota modulation and redox-metabolic pathways, and underscore the value of integrating ancient practices with contemporary biomedical approaches.

Key Words: Yoga, Gut Microbiota, Metabolism, Obesity, Metabolites

2. Introduction

2.1. Background of the Study

Obesity has become one of the most pressing health challenges of our time. By 2022, an estimated 2.5 billion adults were classified as overweight, and nearly 890 million were living with obesity which is roughly 16% of the global adult population. Over the past few decades, the numbers have climbed at an alarming rate; since 1990, obesity levels have more than doubled. If current trends continue, experts predict that by 2050, more than half of all adults—and a third of children—could be affected (*Obesity*, n.d.).

What's especially concerning is the rapid rise of obesity in low- and middle-income countries. These regions are seeing sharp increases but lack adequate healthcare systems to respond to the growing burden. Beyond the physical toll, the financial cost is staggering. By 2035, managing obesity and its consequences could cost the world around USD 4.3 trillion each year—nearly 3% of the global GDP (*Journals | World Obesity Federation*, n.d.).

Today's eating habits, often high in processed foods and low in nutritional value, paired with increasingly inactive lifestyles, have created a setting where people are consuming more calories than they burn. This ongoing imbalance between intake and energy use plays a major role in the rising rates of obesity around the globe. As a result, we're seeing a surge in metabolic disorders that not only affect individual well-being but also pose a growing challenge for public health systems worldwide ("Health Effects of Overweight and Obesity in 195 Countries over 25 Years," 2017).

Obesity doesn't just affect how we look—it deeply interferes with how the body maintains balance in its internal systems. When excess fat builds up, especially around our organs and under the skin, it sets off a slow-burning fire of inflammation throughout the body. This subtle, ongoing inflammation—often referred to as "meta-inflammation"—disrupts the body's metabolic harmony (Tilg & Kaser, 2011).

Fat tissue, once thought to be passive, becomes hormonally active, releasing signalling molecules (cytokines) that can block insulin from doing its job. As a result, the body struggles to regulate sugar levels, leading to insulin resistance and greater metabolic chaos. This imbalance is worsened by immune cells like macrophages flooding fat-rich areas, especially

when the fat cells are too large and oxygen-starved. The tug-of-war between inflammatory and anti-inflammatory immune cells also fuels this dysfunction (Zsálig et al., 2023).

Over time, these disruptions affect how the body handles fats and sugars, often showing up as high triglyceride levels, abnormal cholesterol patterns, elevated blood sugar, and a general decline in the body's ability to manage energy efficiently (Martinez et al., 2016).

Yoga's Emerging Role in Supporting Gut–Metabolism Harmony: Though research directly linking yoga to gut microbiota is still developing, there's growing interest in how this ancient practice may positively influence the intricate relationship between our digestive health and metabolic function.

Echoing the Benefits of Exercise on Microbiome Diversity: Being a holistic way of life, Yoga tend to be more efficient than regular physical activity. It encourages the growth of diverse gut microbes and helps maintain a healthier ratio of Firmicutes to Bacteroidetes—two bacterial groups essential for metabolic regulation. Through its multi-layered approach towards healthy life, yoga can improve digestive flow, reduce inflammation, and foster a more resilient and balanced gut environment.

2.2. Modern Insights into Obesity

Definition and Criteria for Classification

Obesity refers to the **unhealthy buildup of body fat** that can interfere with normal bodily functions and increase the risk of serious health conditions. The World Health Organization (WHO) defines obesity in adults as having a ***Body Mass Index (BMI) of 30 kg/m² or higher,*** a threshold widely used across global populations (*Obesity and Overweight*, n.d.).

However, for ***Asian populations***, this standard has been reconsidered. Research shows that individuals of Asian descent tend to have ***higher body fat percentages and greater abdominal fat*** at lower BMI levels compared to Western populations3. As a result, the ***Asia-Pacific guidelines classify a BMI of 25 kg/m² or more as obese,*** and 23–24.9 kg/m² as overweight, to better reflect the ***increased cardiometabolic risks***—such as diabetes and heart disease seen at these lower BMI values (Cole & Lobstein, 2012; Goda & Masuyama, 2016)

Multifactorial Etiology of Obesity

More than just calories obesity isn't just about eating too much or moving too little—it's a deeply complex and long-lasting condition shaped by many layers of influence. At its centre lies a mismatch between how much energy we consume and how much we expend. But this isn't simply about overeating; many other biological, behavioural, and environmental forces play a role (Hruby & Hu, 2015a).

Modern diets, filled with ultra-processed foods—like those high in refined sugars, unhealthy fats, and salt—make it easy for the body to store excess fat. At the same time, everyday routines have shifted toward less movement: more time sitting in front of screens and fewer opportunities for manual work or active play. This combination leads to an energy surplus that gets stored as fat (Fan et al., 2023).

Determinants and Risk Factors Contributing to Obesity

The development of obesity is influenced by a constellation of interrelated biological, behavioural, and environmental factors that act across the lifespan. One of the most prominent contributors is the increased consumption of ultra-processed foods, which are typically energy-dense and nutrient-poor, often containing excessive amounts of sugar, sodium, and unhealthy fats. These foods are also engineered to enhance palatability and shelf-life, which promotes overconsumption and disrupts satiety signalling (Moradi et al., 2022).

2.3. Gut microbes: Host's Tiny Yet Mighty Partner

Our bodies are filled with trillions of tiny living organisms. Most of them are bacteria, but there are also some viruses, fungi, and other small life forms. These tiny creatures live in different parts of our body and are known as the *microbiota*. The set of all their genes is called the *microbiome* (Lynch & Pedersen, 2016).

Among these, the microbes living in our *gut* are especially important. These *gut microbes* help us break down food, support our immune system, and play a big part in how our body uses and stores energy. Even though they are invisible to the eye, they have a powerful impact on our health—especially on *digestion, immunity, and metabolism* (Valdes et al., 2018a).

2.4. Strategies to Prevent Obesity and its consequences

Prevention of Obesity requires a multi – pronged, evidence-based approach having two phases at the implication level; integrating lifestyle changes at the individual level and policy interventions at the societal level.

At the individual level, life style modifications mainly by adopting whole-food, plant-based diets rich in fruits, vegetables, whole grains, legumes, and nuts, which are associated with reduced body weight and lower risk of chronic disease. Such diets apart from their nutritional benefit, also provide a congenial environment for gut microbial diversity which plays crucial role in deciding metabolic efficiency of the host (Satija et al., 2016).

2.5. Interplay Between Lifestyle, Gut Microbiome, and Metabolic Health

Lately, scientists have discovered that the gut microbiome—the vast community of microbes living in our digestive system—plays a major role in keeping our metabolism and immune system balanced. These tiny organisms, mostly bacteria, number in the trillions and constantly interact with our body's systems. Their influence is especially important when it comes to obesity and long-term diseases linked to it (Lynch & Pedersen, 2016).

2.6. Yoga and Health

Yoga, once revered solely as a spiritual path, is now embraced as a powerful, science-backed approach to holistic health. It nurtures the body and mind through a blend of movement, breath, and awareness—positively influencing systems as diverse as the nervous, endocrine, immune, and digestive networks. Regular practice of yoga has been shown to calm the stress response, balance hormone levels, strengthen immunity, and support gut function, making it a gentle yet effective way to manage lifestyle disorders like obesity and metabolic syndrome. By integrating ancient wisdom with modern biomedical insights, yoga offers a non-pharmacological path to healing—one that honours the interconnectedness of body, mind, and spirit.

2.7. Aim and Objectives of the Study

The central hypothesis of this proposed study is that the practice of yoga has a beneficial effect on the metabolic capacity of the gut microbiome in obese individuals by positively modulating

them. Hence this study focuses on how yoga practice influences the composition and diversity of gut microbiota in obese individuals, as well as a beneficial effect on enhancement of gut microbiota influenced metabolic functions in them. The findings obtained through this study would suggest how effectively the yoga can be used as an effective life-style modality to improve the overall health as well as to reduce the use of medication. Furthermore, this study would justify the beneficial classical references with modern scientific evidence.

The **key questions** expected to be answered by the proposed research are:

1. Do yoga practices have any influence (can modulate/alter) on the composition and diversity of gut microbiota in obese individuals?
2. Whether the practice of yoga has any beneficial effect on enhancement of gut microbiota influenced metabolic functions in obese individuals?

2.8. Aim of the study

To investigate the impact of yoga practices on the composition and diversity of gut microbiota and their downstream influence on host metabolic functions in obese individuals.

Objectives

1 **To assess** the effect of regular yoga practice on the composition, diversity, and relative abundance of gut microbiota in obese individuals.

2 **To evaluate** the influence of yoga-induced microbial changes on host metabolic parameters such as lipid profile, body mass index (BMI), anti-oxidants, oxidative stress markers and insulin.

3 **To explore** the relationship between the duration/intensity of yoga practice, food pattern and the degree of metabolic and microbial modulation.

4 **To compare** baseline and post-intervention data to identify significant changes in metabolic health markers in correlation with gut microbial shifts.

Hypothesis:

Null Hypothesis (H_0):

Yoga practice does not significantly alter the gut microbiota composition or metabolic parameters in obese individuals.

Alternative Hypothesis (H_1):

Regular yoga practice leads to significant modifications in gut microbiota composition and diversity, which in turn positively influence metabolic functions in obese individuals.

3. Review of Literature

Understanding the complex relationship between obesity, metabolism, gut microbiota, and the practice of yoga requires an integrative exploration that spans both ancient wisdom and modern science. While recent advances in microbiology and metabolic health have illuminated the physiological underpinnings of obesity and microbial ecology, ancient Indian yogic texts have long described holistic pathways for maintaining bodily balance, digestion, and well-being through disciplined lifestyle practices. As such, this chapter is organized into two major sections: **Ancient Literature**, which draws upon classical texts and traditional yogic concepts, and **Modern Scientific Findings**, which synthesizes current empirical research in areas such as microbiota, metabolic function, and yoga as a therapeutic intervention.

This dual framework provides a culturally grounded and scientifically validated perspective, aiming to bridge time-honoured yogic traditions with contemporary biomedical research. By weaving together these streams of knowledge, the review seeks to construct a multidimensional understanding of how yoga may beneficially influence gut microbiota and metabolism in the context of obesity.

3.1. Ancient Literature

The ancient wisdom of Ashtangayoga, Hatha Yoga, Ayurveda, and associated philosophical systems offers a profoundly holistic framework for understanding digestion, metabolism, and internal health. These traditions interlace principles of diet, cleansing, physical and energetic practice, and mind-body harmony—providing invaluable context for modern inquiries into gut health and metabolic regulation.

3.2. Foundational Yogic and Ayurvedic Concepts Defining Individual Health and Their Relationship with Metabolism

Ayurveda and Yoga, though developed as distinct disciplines, share a deeply interconnected vision of health that goes far beyond simply avoiding illness. In both traditions, true well-being—referred to as *swasthya*—is seen as a dynamic balance between the body (*sharira*), mind (*manas*), senses (*indriyas*), and consciousness (*atman*) (Sharma et al., 2014). Ayurveda defines a healthy person as one whose biological energies (*doshas*), digestive fire (*agni*), bodily tissues (*dhatus*), and waste elimination (*malas*) are in harmony, and whose inner faculties are content and clear (Keval Krishna Thakral, 2017). Yoga complements this view by emphasizing

mental steadiness, clarity, and self-awareness as essential foundations for physical health, as outlined in Patanjali's Yoga Sutras. Together, these systems offer a holistic framework for living with balance, vitality, and inner peace (Vivekānanda, 2006).

3.3. Dhatu: The Seven Bodily Tissues

Ayurveda describes seven core tissues—called *dhatus*—that form the foundation of our physical body, literally these 'hold' the body together: *Rasa* (plasma and lymph), *Rakta* (blood), *Mamsa* (muscle), *Meda* (fat), *Asthi* (bone), *Majja* (marrow and nervous tissue), and *Shukra/Artava* (reproductive tissue). These layers are nourished in a step-by-step process, where each tissue draws its strength from the one before it, guided by specific metabolic energies known as *Dhatvagnis* (Agrawal et al., 2010; Keval Krishna Thakral, 2017; Sharma et al., 2014).

From a modern perspective, these dhatus can be seen as the body's functional compartments—each relying on proper digestion, nutrient absorption, and circulation to stay healthy. When this balance is disturbed, it can lead to either depletion (*kshaya*) or excess (*vriddhi*) of tissues, which may result in various health issues, including metabolic disorders

A yogic lifestyle naturally supports the nourishment and balance of these tissues. Practices like mindful eating (*mitahara*), regular practice, and adequate rest help the body digest food efficiently and distribute nutrients where they're needed most. By living in tune with these principles, we not only care for our physical structure but also support deeper vitality and resilience (Bhat, 2006).

3.4. Mental Well-being and Metabolic Health

Indian systems like Yoga and Ayurveda recognises that mental health is inseparable from physical health. The **three gunas**—*Sattva* (clarity), *Rajas* (activity), and *Tamas* (inertia)—govern mental states and behaviour (Bhat, 2006). Psychological disturbances (*manasika vikara*) are often both a cause and consequence of metabolic imbalance (Keval Krishna Thakral, 2017).

Modern science supports this bidirectional link: chronic stress and emotional dysregulation alter hypothalamic–pituitary–adrenal (HPA) axis activity, influence gut microbiota, and disrupt glucose and lipid metabolism (Kiecolt-Glaser et al., 2015). Yogic meditation, mindfulness, and

breath regulation are known to modulate the autonomic nervous system, improve gut-brain axis communication, and enhance metabolic resilience (Pascoe, Thompson, & Ski, 2017).

3.5. Food and Digestion in Haṭha Yoga

The *mitāhāra* concept is central. *Haṭha Pradīpikā* (1.57–1.59) defines *mitāhāra* as wholesome, moderate, and sattvic food, taken in the right quantity, leaving one-quarter of the stomach empty to aid digestion. Prohibited foods (*apāthya*) include stale, overly spicy, excessively oily, or overly fermented items, which are believed to obstruct *nāḍīs* and generate *āma* (Digambarji & Jha, 1998). The same is explained in *Gheranda Samhita*, another prominent Hatha Yoga text.

- **Pathya (wholesome)**: fresh vegetables, whole grains, pulses, milk, ghee in moderation, fresh fruits.
- **Apāthya (unwholesome)**: meat (especially heavy, stale), alcohol, fried foods, excess sweets, and highly processed items.
- **Mitāhāra**: mindful eating with focus on satiety, not fullness.

3.6. Gut Bacterial Taxa and Their Effects on Host Metabolism and Obesity

The human gastrointestinal tract harbours a highly diverse and dynamic community of microorganisms, dominated primarily by the phyla Firmicutes, Bacteroidetes, Actinobacteria, and Proteobacteria. Shifts in the composition and function of these microbial communities—termed gut dysbiosis—have been strongly associated with metabolic disorders, including obesity, insulin resistance, and dyslipidemia (Rinninella et al., 2019; Turnbaugh et al., 2006e). Notably, obesity is often characterized by a higher Firmicutes-to-Bacteroidetes ratio, although this pattern is not universal across all studies (Magne et al., 2020a). Below, major bacterial taxa implicated in metabolic regulation and obesity are discussed.

3.7. Research Gap

While diet, exercise, bariatric surgery, and targeted microbial therapies have well-documented effects on gut health and metabolism, there's a lack of rigorous studies that isolate yoga as a standalone intervention and simultaneously measure its impact on the gut microbiome, metabolites (like SCFAs and bile acids), gut permeability markers (such as LPS and zonulin), stress hormones (like cortisol), stress markers, anti-oxidants, oxidative stress markers, and

clinical outcomes like blood sugar and lipid levels. Without such data, we can't yet say for sure whether yoga can directly reshape the gut–microbiome–metabolism axis in people with obesity.

So, studying yoga's impact on the gut microbiome and metabolism doesn't just fill a gap in obesity research—it also builds a bridge between ancient wisdom and modern science. If the study shows that yoga can influence metabolism through changes in the microbiome, it would offer scientific support for age-old claims about yoga's digestive and metabolic benefits. This could help bring yoga into mainstream healthcare as a respected, evidence-based approach to managing metabolic disorders.

4. Methodology

4.1. Study Design, Participants and Location

This study followed a basic two-group design using simple random sampling. A total of 23 participants were included: **16 in the Yoga Group (YG)** and **7 in the Control Group (CG)**. Both groups were matched for age, body weight, and BMI to ensure fair comparison at the start.

The research was carried out at the **Department of Human Consciousness & Yogic Sciences, Mangalore University**, in partnership with the **Nitte University Centre for Science Education and Research (NUCSER), Mangalore**.

4.2. Eligibility Criteria

Age and Sex Eligibility

- Age: **18–50 years**
- Sex: **Both male and female participants**

Inclusion Criteria

- Participants with **BMI ≥ 25.0 kg/m²** (classified as obese according to the **Asian Pacific Classification of Obesity**)
- Both male and female individuals fulfilling the BMI criterion

Exclusion Criteria

- Pregnant or lactating women
- Individuals with cardiovascular disease, liver or kidney function impairment
- Participants with HCV, HIV, or HBsAg seropositive status
- Patients with known gastrointestinal bleeding
- Psychologically ill individuals

- Individuals under the age of 18 years

4.3. Recruitment and Screening Process

Participants were recruited through the Yoga Therapy Centre at Mangalore University, (Mangalagangotri Campus) and via local advertisements. Interested individuals underwent a telephone interview to obtain preliminary details and were subsequently invited for an in-person medical examination, which included:

- Detailed case history assessment
- Physical examination
- Review of medical and medication history

Eligible participants, as per predefined criteria, received comprehensive written and verbal information about the study. Written informed consent was obtained before enrolment.

Between March 2021 and December 2022, 40 individuals were screened; 23 met the inclusion criteria and were randomly assigned to the YG (n = 16) or CG (n = 7).

4.4. Ethical Approval and Registration

The study was approved by the **Institutional Human Ethics Committee (IHEC)** of Mangalore University (Approval No.: MU-IHEC-2021-1).

4.5. Validation of Protocol and Intervention Details

To ensure the scientific rigor and clinical relevance of the yoga module designed for the study *"Effect of Yoga on Gut Microbiota Influenced Immunomodulatory Functions in Obese Individuals"*, a systematic validation process was undertaken. A panel of experienced yoga experts was invited to critically review the module's structure, content, and applicability. Their evaluation was facilitated through standardized tools, including a structured scoring sheet and a "Yoga Performance Assessment" document, enabling detailed and objective feedback. Accompanying materials—such as the study's aims and objectives, along with classical yogic references that guided the module's development—were provided to inform their assessment.

This collaborative process not only refined the sequence and progression of practices but also reinforced the interdisciplinary approach of the research, highlighting the integration of

traditional wisdom with modern evidence-based methodology in obesity management. The validated yoga protocol incorporated a balanced combination of *asanas* (postures), *pranayama* (breathing techniques), *bandhas* (energy locks), *mudras* (gestures), relaxation methods, and *kriyas* (cleansing practices). The intervention was delivered to the Yoga Group (YG) with careful attention to individual needs and physical capabilities. Special accommodations were made for female participants during menstruation, during which they abstained from *asanas* for five days and instead practiced *pranayama* and guided relaxation techniques, ensuring both safety and comfort throughout the intervention period.

4.6. Total duration in weeks/months for yoga therapy

Yoga practice will be taught by a qualified yoga instructor at the Yoga Therapy Centre of the Department of Human Consciousness & Yogic Sciences, Mangalore University. The practice session with specific module consists of 60 minutes Yoga Therapy intervention for for 10 weeks.

4.7. Study Parameters and Collection of Samples

Anthropometric and blood biochemical estimation

Prior to the commencement of the intervention, a comprehensive set of baseline data was collected from all participants to ensure comparability between the Yoga Group (YG) and Control Group (CG). Anthropometric measurements included body weight (in kilograms), standing body height (in centimetres), and body mass index (BMI) calculated as weight in kilograms divided by the square of height in meters (kg/m^2). Waist circumference (in centimetres) was measured at the midpoint between the lower margin of the last palpable rib and the top of the iliac crest, providing an indicator of central adiposity. Resting physiological parameters were also assessed; systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured in the brachial artery using an oscillometric device, following standard clinical protocols to ensure accuracy and reproducibility.

In addition to anthropometric and physiological data, fasting blood samples were collected from each participant to assess biochemical markers relevant to metabolic health and inflammation. These included fasting blood glucose, total cholesterol (TC), high-density lipoprotein cholesterol (HDLc), low-density lipoprotein cholesterol (LDLc), and triglycerides (TG). To explore oxidative stress and antioxidant defence status, vitamin C, reduced

glutathione (GSH), oxidized glutathione (GSSG), Insulin and malondialdehyde (MDA) levels were measured. All those levels were quantified using commercially available Human GenELISA Kits (KrishGen Biosystems Ltd., India). For these analyses, fasting venous blood was drawn in the morning after an overnight fast. Samples collected in EDTA-treated vials were processed immediately; one aliquot of whole blood was treated with equal volumes of 10% metaphosphoric acid and 10% trichloroacetic acid (TCA) to stabilize antioxidants for subsequent biochemical estimation.

4.8.Gut Microbiota Analysis

Sample Collection:

- Faecal samples were collected at **baseline** and **post-intervention**
- Samples were delivered to the lab within 30 minutes of defecation

DNA Extraction and Sequencing:

16S rRNA-Based Metagenomic Sequencing

In sterile laboratory conditions, over 20 mg of each faecal sample was used for DNA extraction. The extraction was performed using the commercially available QIAgen® DNeasy Blood and Tissue Kit (Cat. #51306, QIAGEN, Germany). The quality and quantity of the extracted DNA were assessed using a Nanophotometer® NP80 (IMPLEN, Germany), ensuring a minimum concentration of 50 ng/µL and a purity ratio (A260/A280) of 1.8. Following quantification, the DNA samples were stored at –20°C until further processing. High-throughput sequencing targeting the V3–V4 hypervariable regions of the bacterial 16S rRNA gene was subsequently carried out at Biokart India Pvt. Limited, utilizing the Illumina sequencing platform to generate paired-end reads.

Analyses of the Gut Microbiome

The raw sequencing reads were initially pre-processed and quality-filtered using Trim Galore v0.6.2, followed by analysis through the QIIME platform available on the NCBI Nephele server. The quality of the filtered reads was evaluated using FastQC v0.11 to confirm their suitability for downstream processing. Quality-approved paired-end reads, along with the corresponding metadata files, were subsequently analysed using the QIIME 2 pipeline to conduct detailed statistical, functional, and integrative assessments of the microbiome data.

Prior to taxonomic and diversity analyses, the forward and reverse reads were merged into contigs using default parameters. Taxonomic classification was performed with VSEARCH, and sequences were clustered against a closed-reference OTU database at 97% sequence identity. The resulting BIOM files generated from QIIME 2 were then imported into the MicrobiomeAnalyst platform—an open-access, web-based tool—for downstream statistical exploration and interpretation of microbial composition.

The analyses included comparisons of microbial diversity before and after the yoga intervention in obese participants. Alpha diversity, representing the richness and evenness of operational taxonomic units (OTUs) within the samples, was quantified using indices such as Observed Species, Shannon Index (richness), and Simpson Index (evenness). Group-wise comparisons were performed using the Mann–Whitney U test, with statistical significance set at $p < 0.005$.

Beta diversity, reflecting differences in microbial composition between samples, was assessed using the Permutational Multivariate Analysis of Variance (PERMANOVA) test, along with Bray–Curtis dissimilarity metrics to construct a Principal Coordinate Analysis (PCoA) plot. Finally, enrichment analysis of differentially abundant bacterial genera was carried out using the Linear Discriminant Analysis Effect Size (LEfSe) method to identify taxa most strongly associated with the pre- and post-intervention states.

4.9.Statistical Analysis

In the statistical analysis of this thesis, GraphPad Prism software served as the primary tool for conducting various analyses. Paired t-tests were employed to compare pre and post-intervention measurements within each group, assessing changes over time. Unpaired t-tests, on the other hand, were utilized to compare changes between the yoga and control groups, enabling the identification of differences in outcomes between the two interventions. The presentation of data, measurements, and calculated values was conducted with meticulous attention to detail, ensuring clarity and accuracy in reporting. Descriptive statistics, such as means ± standard deviation or medians, were utilized to provide a comprehensive overview of the data distribution. Additionally, specialized bioinformatics tools such as Microbiome Analyst were employed to extract genomic data and comprehensively analyze gut bacteria fold changes, facilitating a deeper understanding of the microbial dynamics influenced by the interventions.

5. Results

5.1. Summary of Collected Data

This chapter provides a detailed account of the data obtained during the study, covering multiple parameters relevant to the research objectives. It begins with a description of the baseline characteristics of the participants belonging to two groups Yoga Group (YG) which also the experimental group and the Control Group (CG) which is non-yoga control, establishing a clear profile of the study population. The subsequent sections present statistical findings, highlighting changes observed in anthropometric, physiological, biochemical, metabolic markers, and gut microbiota measures. Through this systematic analysis, the results aim to illustrate the influence of yoga on a wide spectrum of physical and physiological indicators, offering a comprehensive perspective on its potential benefits.

5.2. Baseline Characteristics of the Study Participants

Establishing a clear baseline is a vital step in interpreting the outcomes of any intervention, as it provides a reference framework against which post-intervention changes can be evaluated. This section outlines in detail the initial characteristics of the participants enrolled in the study, highlighting their demographic, anthropometric, and physiological profiles prior to the commencement of the yoga intervention. These data serve as an essential benchmark, enabling a meaningful comparison of pre- and post-intervention outcomes, and ensuring that any observed changes can be attributed to the intervention rather than pre-existing differences.

The baseline data of the 23 participants, divided into the Obese Yoga Group (YG; n = 16) and the Obese Control Group (CG; n = 7), are presented in Table 3.

Table 3: *Baseline assessment of the demographic, anthropometric, and physiological profiles*

Sl. No.	**Anthropometric Parameters**	**Obese Yoga Group (n=16)**	**Obese Control group (n=7)**
1.	Age (years)	033.4 ± 5.4	025.14 ±2.116
2.	Gender (F/M)	9 / 7	3 / 4
3.	Height (m)	0162.2 ± 9.379	0169.0 ± 12.42

Sl. No.	Anthropometric Parameters	Obese Yoga Group (n=16)				Obese Control group (n=7)			
		Median	***Mean ± SD***	***t***	***p***	***Median***	***Mean ± SD***	***t***	***p***
4.	Weight (Kgs)	075.45	076.21 ±9.706	031.41	<0.0001	091.6	088.63 ±15.78	014.86	<0.0001
5.	BMI (Kg/m^2)	028.7	028.91 ±2.703	042.78	<0.0001	029.6	30.96 ±4.453	018.39	<0.0001
6.	WC (cm)	038	038.94 ±2.955	052.72	<0.0001	040	41.14 ±3.388	032.13	<0.0001
7.	HC (cm)	042	042.06 ±3.043	055.28	<0.0001	044	43.71 ±4.786	024.17	<0.0001
8.	S. B. P. (mmHg)	0120	0119.1 ±8.702	054.76	<0.0001	0126	122.9 ±6.914	047.01	<0.0001
9.	D. B. P. (mmHg)	080	081.00 ±6.491	049.92	<0.0001	80	80.29 ±10.55	020.14	<0.0001

In terms of age, YG participants recorded a median of 033.5 years and a mean of 033.4 ± 5.4, showing a statistically significant difference ($t = 024.54$, $p < 0.0001$) compared to CG, whose median age was 25 years with a mean of 25.14 ± 2.116. Regarding weight, YG had a median of 75.45 kg and a mean of 76.21 ± 9.706, significantly lower ($t = 31.41$, $p < 0.0001$) than CG, which showed a median weight of 91.6 kg and a mean of 88.63 ± 15.78.

For Body Mass Index (BMI), YG displayed a median of 028.7 and a mean of 028.91 ± 2.703, with a highly significant difference ($t = 42.78$, $p < 0.0001$) compared to CG, which had a median BMI of 029.6 and a mean of 030.96 ± 4.453. Waist circumference (WC) in YG was 038 cm (median) and 38.94 ± 2.955 (mean), while CG recorded 40 cm (median) and 041.14 ± 3.388 (mean), again with significant differences. Hip circumference (HC) followed a similar trend, with YG showing 042 cm (median) and 042.06 ± 3.043 (mean), compared to CG's 44 cm (median) and 043.71 ± 4.786 (mean).

Blood pressure measures also varied between groups. Systolic Blood Pressure (SBP) in YG was 120 mmHg (median) and 119.1 ± 8.702 (mean), whereas CG recorded 126 mmHg (median) and 0122.9 ± 6.914 (mean). Diastolic Blood Pressure (DBP) in YG was 80 mmHg (median) and 081.0 ± 6.491 (mean), compared to CG's 80 mmHg (median) and 080.29 ± 10.55 (mean).

Overall, these baseline findings highlight measurable differences between the YG and CG in several anthropometric and physiological parameters. This initial profile is critical for interpreting the forthcoming results and for assessing the true impact of yoga on obesity-related health markers.

5.3. Statistical Analysis of Variations in Anthropometric, Physiological, and Blood Biochemical Markers

Anthropometric Measurements

Anthropometric measurements provide essential insights into the physical profile and overall health status of the participants at the outset of the study. In this research, parameters such as age, body weight, Body Mass Index (BMI), waist circumference (WC), hip circumference (HC), and both systolic and diastolic blood pressure were carefully documented. These measurements are widely recognized as reliable indicators for evaluating body composition, fat distribution, and cardiovascular risk.

By establishing precise baseline values, the study ensures a robust framework for tracking changes resulting from the yoga intervention. For example, reductions in body weight or BMI can reflect improved metabolic efficiency, while changes in WC and HC may indicate shifts in visceral and peripheral fat distribution. Monitoring blood pressure trends provides further insight into potential cardiovascular benefits. Together, these anthropometric indicators allow for a comprehensive evaluation of how yoga practice may contribute to weight management, improved body proportions, and better physiological regulation in obese individuals.

Table 4: *Paired t-test Comparison of Post–Pre Scores for Anthropometric Parameters in the Yoga Group (YG) and Control Group (CG)*

Paired t-test Yoga and Control Group Post-Pre	**Mean**	**SD**	**SEM**	**95% confidence interval**		**t**	**df**	**P value (Two tailed)**	**P = < 0.05?**
				Lower	Upper				
BMI YG	-01.638	0.3052	0.07631	-1.800	01.475	21.46	015	<0.0001	Yes
BMI CG	0.37	0.45	0.17	-0.041	0.78	2.2	06	0.0698	No
Weight YG	-04.288	0.9091	0.2273	-4.772	03.803	018.86	015	<0.0001	Yes
Weight CG	0.9571	1.218	0.4603	-0.1691	02.083	02.080	06	0.0828	No
W/H YG	-0.025	0.01033	0.00258	-0.0305	0.01950	09.682	015	<0.0001	Yes
W/H CG	0.00571	0.01397	0.00528	-0.0072	0.01864	01.082	06	0.3208	No

Weight:

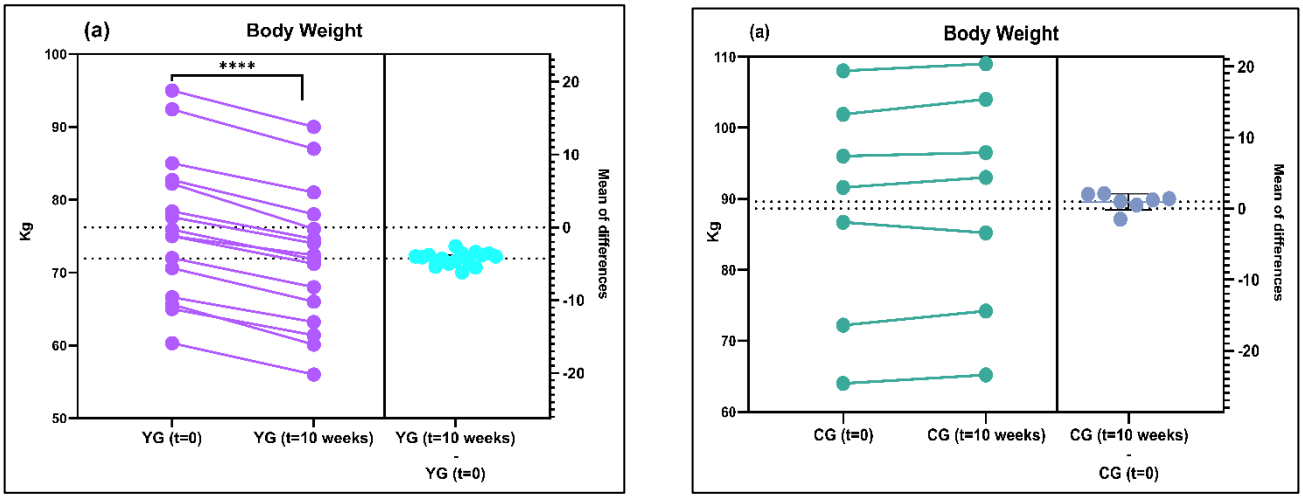


Figure 2: *Estimation plots illustrating pre- and post-intervention changes in body weight for the Control Group (CG-Right Plot) and Yoga Group (YG-Left Plot) over a 10-week period.*

(a) ***Yoga Group (YG)*** *– The left panel presents paired weight (Kgs) measurements at baseline (t=0) and after the 10-week yoga program, showing a consistent downward trend across participants. The right panel depicts the mean weight difference with 95% CI, highlighting a statistically significant reduction in body weight following the intervention (**** p < 0.0001).*

(a) ***Control Group (CG)*** *– The left panel shows individual paired weight measurements at baseline (t=0) and after 10 weeks (t=10 weeks), connected by lines. Minimal variation is observed between pre- and post-intervention values. The right panel displays the mean difference in weight, with error bars representing the 95% confidence interval (CI), indicating no statistically significant change.*

The weight analysis showed a significant reduction in the Yoga Group (M = -04.288, SD = 00.9091, SEM = 00.2273, 95% CI [-04.772, -03.803], t = 018.86, df = 15, p < 0.0001), indicating notable weight loss following the intervention. However, the Control Group displayed no statistically meaningful change in weight (M = 00.9571, SD = 01.218, SEM = 00.4603, 95% CI [-00.1691, 02.083], t = 02.080, df = 6, p = 0.0828), suggesting that weight remained relatively stable without the yoga program.

BMI:

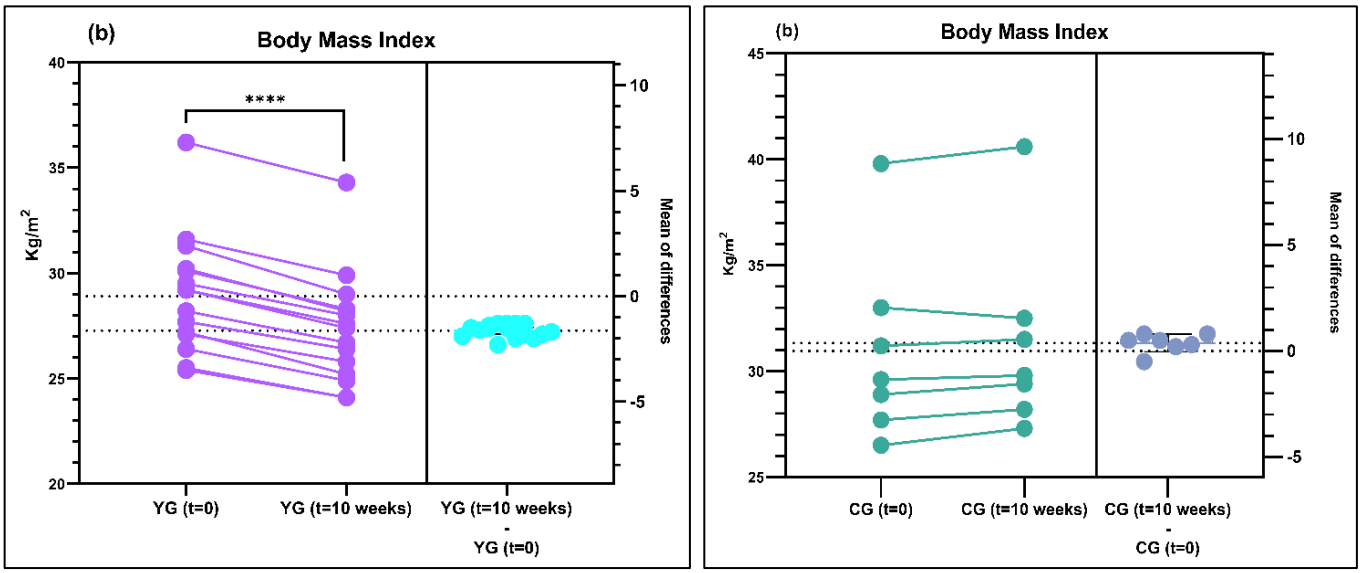


Figure 3: *Pre- and post-intervention changes in Body Mass Index (BMI) within the Yoga Group (Left plot) and Control group (Right Plot).*

The paired plots illustrate BMI measurements at baseline (t = 0) and after 10 weeks (t = 10 weeks) for the Yoga Group (YG) and Control Group (CG). In both the plots, each dot

represents an individual participant's BMI at the respective time point, while connecting lines link paired values, indicating within-subject changes over the intervention period.

A paired t-test was performed to assess the variation in Body Mass Index (BMI) from pre- to post-intervention in both the Yoga Group (YG) and Control Group (CG). For the YG, results demonstrated a marked and statistically significant decline in BMI (M = -01.638, SD = 00.3052, SEM = 00.07631, 95% CI [-01.800, -01.475], t = 021.46, df = 015, p < 0.0001), highlighting a considerable reduction attributable to the yoga intervention. In contrast, the CG exhibited no statistically significant difference in BMI over the same period (M = 00.37, SD = 00.45, SEM = 00.17, 95% CI [-00.041, 00.78], t = 02.2, df = 06, p = 00.0698), indicating that BMI levels remained largely unchanged without the yoga regimen.

Waist/Hip Ratio:

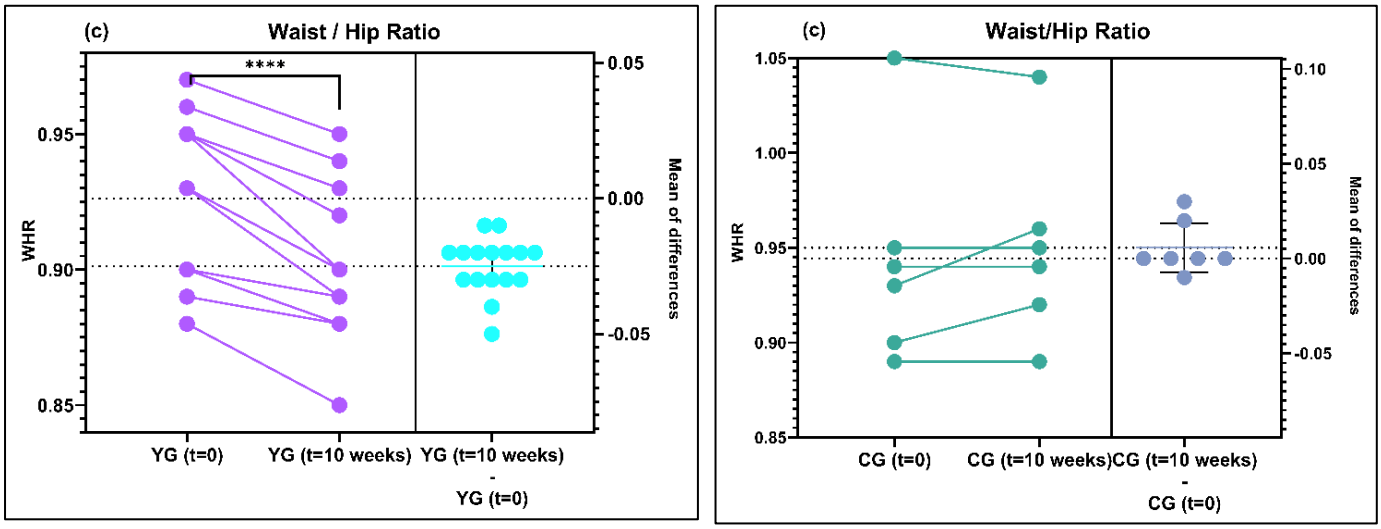


Figure 4: *Pre- to Post-Intervention Changes in Waist-to-Hip Ratio (WHR) in Yoga (left plot) and Control Groups (right plot).*

The left panels in each plot depict individual WHR values at baseline (t = 0) and after 10 weeks (t = 10 weeks) for the Control Group (CG) and Yoga Group (YG), respectively. In the CG plot, green markers represent each participant, with paired measurements connected by lines to illustrate within-subject changes, showing minimal variation between time points. In contrast, the YG plot displays purple markers with connecting lines that predominantly slope downwards, indicating a reduction in WHR following the yoga intervention. The right panels present the distribution of mean differences (post–pre) for each participant in both groups. In the CG, blue points cluster around zero, suggesting negligible overall change. However, in the

*YG, cyan points are consistently below zero, reflecting a notable decrease in WHR. Statistical analysis reveals no significant mean difference in the CG, whereas the YG exhibits a highly significant reduction (****, $p < 0.0001$), underscoring the potential effect of yoga practice on central adiposity reduction.*

In the Yoga Group, the Waist/Hip ratio showed a statistically significant reduction (M = -0.025, SD = 00.01033, SEM = 0.00258, 95% CI [-0.0305, -0.01950], t = 09.682, df = 15, $p < 0.0001$), suggesting that yoga had a favourable influence on this anthropometric measure. In contrast, the Control Group demonstrated no meaningful change in Waist/Hip ratio (M = 0.00571, SD = 00.01397, SEM = 00.00528, 95% CI [-00.0072, 0.01864], t = 01.082, df = 06, p = 00.3208), indicating the absence of a significant effect over the 10-week period.

Post Interventional Comparison: Yoga vs. Control Group After 10 Weeks

Post-intervention, unpaired t-tests were performed to compare anthropometric measures between the Yoga Group (YG) and the Control Group (CG). Findings indicated markedly greater improvements in the Yoga Group.

BMI: Participants in the Yoga Group had a significantly lower BMI than those in the Control Group (t = 02.798, df = 021, p = 00.0108). The mean BMI was 027.27 ± 1.451 for the Yoga Group and 031.33 ± 6.914 for the Control Group, reflecting a mean difference of -04.060 (95% CI: -07.078 to -01.042).

Weight: The Yoga Group experienced a notably greater reduction in body weight compared to the Control Group (t = 03.360, df = 021, p = 00.0030). Mean weight in the Yoga Group was 071.92 ± 05.258, whereas in the Control Group it was 089.59 ± 010.55, yielding a mean difference of -017.67 (95% CI: -028.60 to -06.731).

WHR: Waist-to-Hip Ratio was significantly lower in the Yoga Group relative to the Control Group (t = 03.224, df = 021, p = 00.0041). The Yoga Group recorded a mean WHR of 00.9013 ± 00.01512, compared to 00.9500 ± 00.01512 in the Control Group, corresponding to a mean difference of -00.04875 (95% CI: -00.08019 to -00.01731).

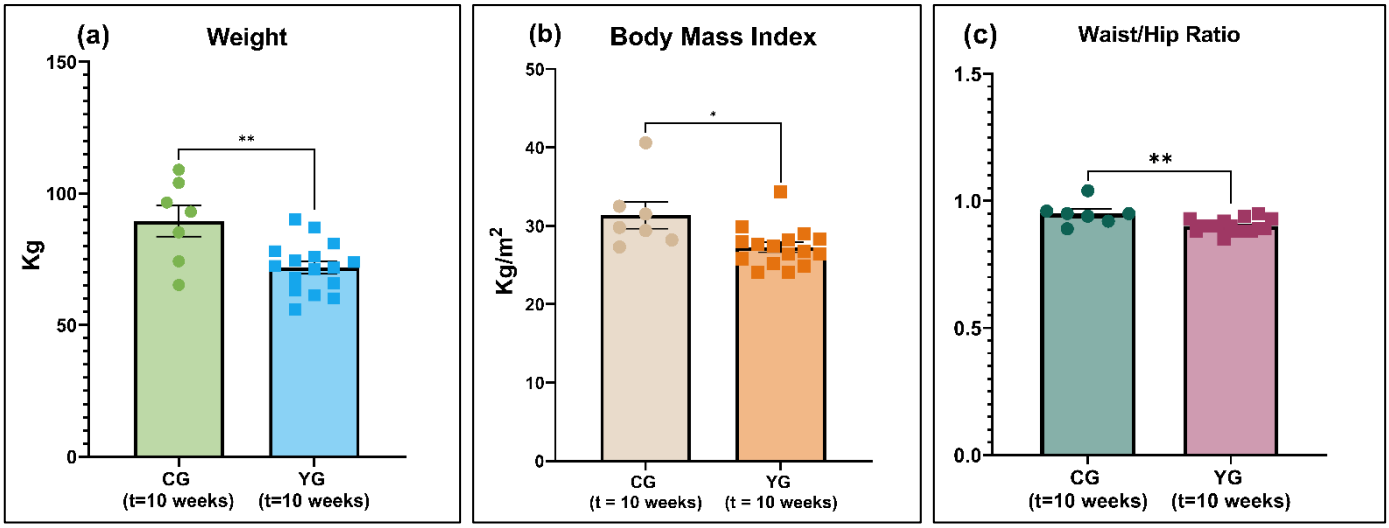


Figure 5: *Comparison of Weight, BMI and Waist-to-Hip Ratio between Control Group (CG) and Yoga Group (YG) after 10 Weeks.*

*The bar charts illustrate post-intervention measurements for the Control Group (CG) and Yoga Group (YG) at t = 10 weeks. In the first plot, representing body weight, the green bar denotes CG values, while the blue bar indicates YG values. A clear reduction in mean body weight is evident in the YG compared to the CG, with statistical significance (**$p < 0.001$**), as marked by the double asterisk above the bars. The second plot presents BMI data, with the CG shown in Grey and the YG in orange. The YG demonstrates a noticeably lower mean BMI than the CG, with achieving statistical significance (**$p < 0.01$**). The third plot presents waist-to-hip ratio data, with the CG shown in teal and the YG in rose-pink. The YG demonstrates a noticeably lower mean WHR than the CG, again achieving statistical significance (**$p < 0.001$**).*

In all the three charts, individual data points are overlaid on the bars to depict participant-level variation, and horizontal connecting lines highlight the between-group comparison. These results reinforce that the yoga intervention was associated with more favourable anthropometric outcomes compared to no intervention.

5.4. Blood Biochemical Assessment

Evaluating blood biochemical parameters — including lipid profile, blood glucose, and other key metabolic markers — offers valuable insight into participants' internal physiological status. This section examines the alterations in these indicators following yoga practice, highlighting its potential role in promoting metabolic equilibrium and reducing obesity-related risk factors.

Total Cholesterol:

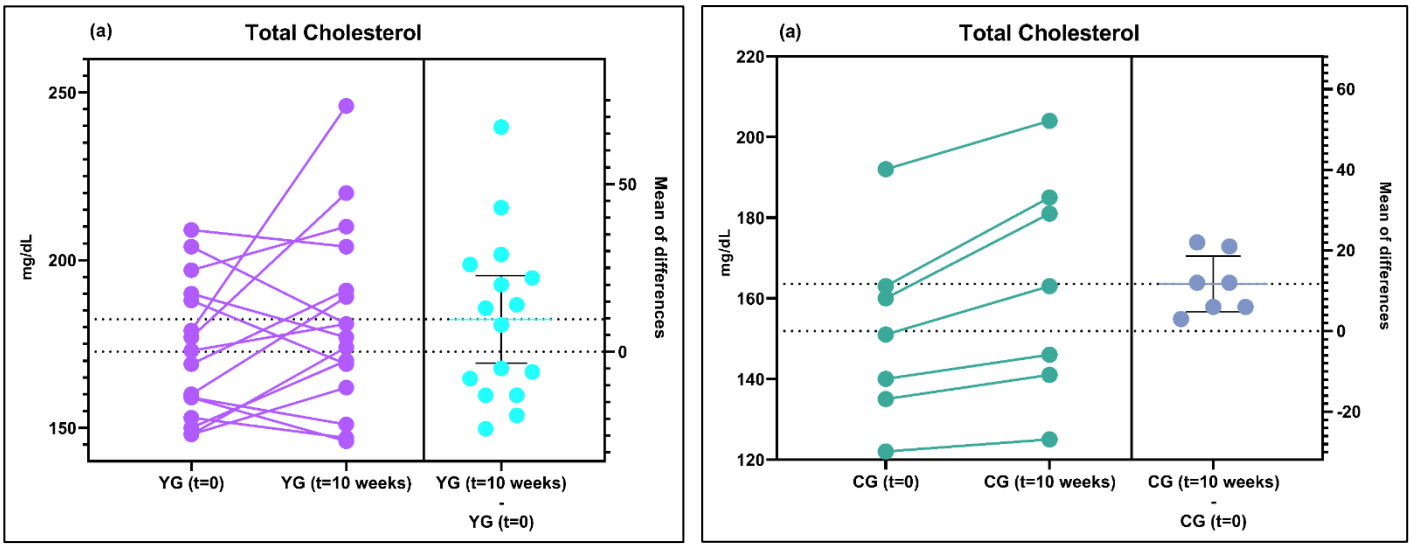


Figure 6: *Estimation plots showing paired t-test analysis of total cholesterol levels in yoga group (YG) and control group (CG) over a 10-week of Yoga intervention.*

The visualization employs distinct color coding to differentiate between study groups, with teal markers and trajectories representing the control group participants, while purple indicators and connecting paths denote the yoga intervention group subjects.

Initial measurements (t=0) establish pre-intervention cholesterol baselines, where control group values range from approximately 120-195 mg/dL with relatively tight clustering, whereas the yoga group exhibits broader baseline variation spanning 145-210 mg/dL. Post-intervention assessments (t=10 weeks) capture cholesterol levels following the study period, revealing contrasting patterns between groups.

Individual trajectory lines map each participant's cholesterol progression throughout the intervention duration. In the control group, these connectors predominantly display upward slopes, indicating cholesterol elevation across most subjects. The yoga group presents more complex trajectory patterns, with crossing lines revealing mixed responses - some participants experiencing reductions while others show increases or stability.

Mean difference statistical significance appears in the estimation panels on the right. The control group demonstrates a positive mean difference with confidence intervals suggesting statistically significant cholesterol increases. In contrast, the yoga group's effect size visualization indicates minimal average change with confidence intervals spanning zero,

suggesting no significant overall cholesterol modification despite considerable inter-individual variation in treatment response.

No statistically significant change was observed in the Yoga Group (M = 09.688, SD = 024.52, SEM = 06.130, 95% CI: –03.379 to 022.75; t = 01.580, df = 015, p = 00.1349). In contrast, the Control Group demonstrated a significant rise in total cholesterol levels (M = 011.71, SD = 07.455, SEM = 02.818, 95% CI: 04.820 to 018.61; t = 04.158, df = 06, p = 00.0060). These findings highlight the need for further research to determine whether the Yoga Group experiences more favourable changes compared to the Control Group.

Triglyceride:

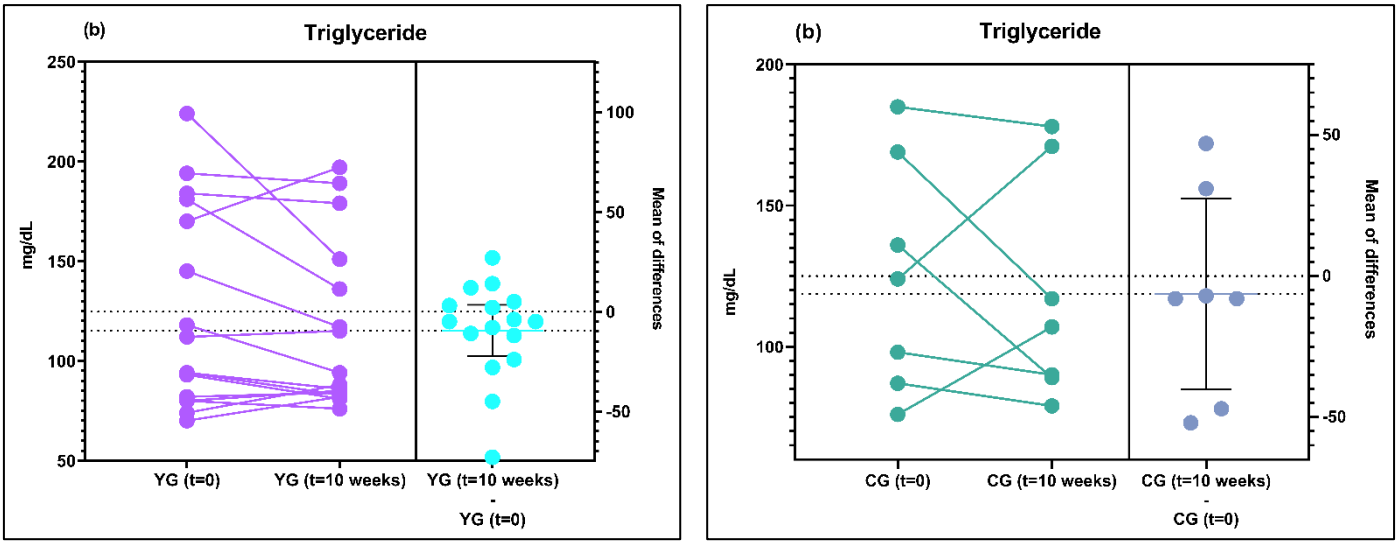


Figure 7: *Paired t-test estimation plots demonstrating triglyceride level changes in yoga group (YG) and control group (CG) over a 10-week intervention period.*

Purple lines and data points denote (left plot) the yoga intervention group, characterized by higher baseline triglyceride concentrations spanning 70-220 mg/dL. The purple trajectory lines demonstrate more pronounced downward slopes across multiple participants, suggesting systematic triglyceride reduction following the yoga intervention.

Teal lines and markers represent (right plot) the control group participants, displaying baseline triglyceride values ranging from 75-185 mg/dL at study initiation. These teal connecting lines exhibit predominantly horizontal or slightly declining trajectories, with some participants showing crossing patterns that indicate varied individual responses without consistent directional change.

Statistical significance interpretation emerges from the estimation plots positioned on the right panels. The control group's effect size visualization shows confidence intervals hovering around zero with modest negative deviation, indicating minimal triglyceride changes that lack statistical significance. Conversely, the yoga group's estimation plot reveals confidence intervals positioned below zero, suggesting meaningful triglyceride reductions with potential clinical significance.

The contrasting colour patterns effectively illustrate intervention efficacy, where teal trajectories remain relatively stable while purple pathways demonstrate beneficial lipid profile improvements through yoga practice implementation over the study duration.

PPBS (Postprandial Blood Sugar):

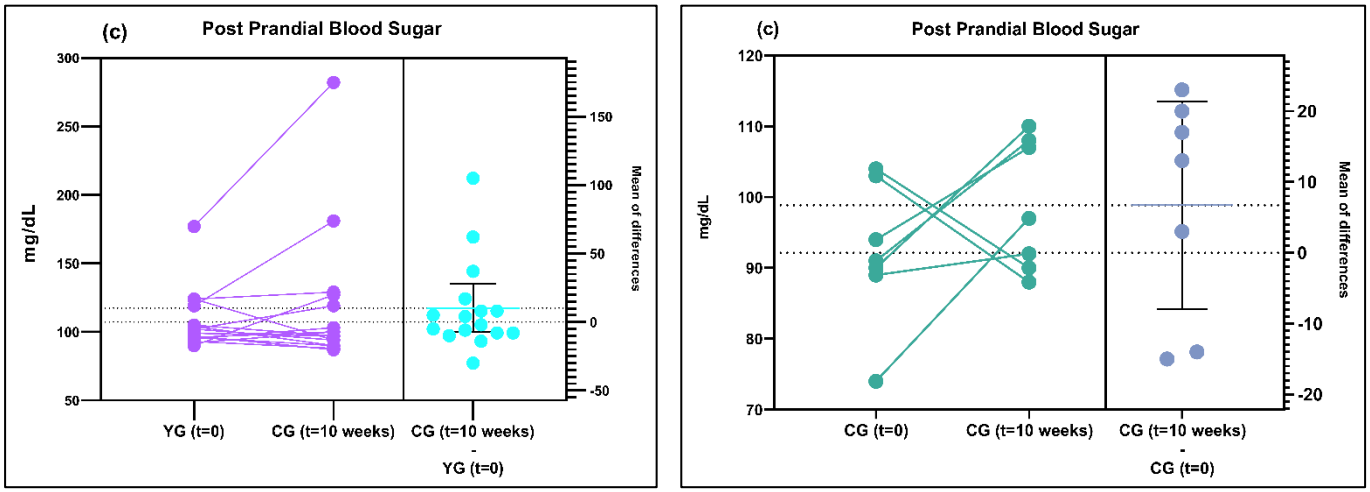


Figure 8: *Comparative analysis of postprandial blood sugar changes in yoga group (YG) and control group (CG) over a 10-week intervention period*

Left plot represents the trend of PPBS within the Yoga Group in purple lines and Right plot is of Control Group with green trend line showing the change in PPBS during the course time.

The visualization demonstrates paired measurements of postprandial blood glucose levels across two distinct study populations. Baseline measurements (t=0) represent initial blood sugar values before any intervention, with the control group showing values clustered around 90-105 mg/dL and the yoga group displaying a wider distribution ranging from approximately 90-180 mg/dL. Follow-up assessments (t=10 weeks) capture glucose levels after the completion of the intervention period, revealing varied responses between the two cohorts.

Connecting lines trace individual participant trajectories, illustrating the direction and magnitude of change for each subject throughout the study duration. These trajectory indicators

provide insight into intervention responsiveness at the individual level, with some participants showing improvement while others demonstrate stability or deterioration.

Statistical significance of mean differences is represented through the right-side panels displaying effect sizes with confidence intervals. The control group exhibits minimal change with tight confidence intervals around zero, suggesting no meaningful alteration in postprandial glucose levels. Conversely, the yoga intervention group demonstrates considerable variability in treatment response, with the mean difference confidence interval indicating potential beneficial effects, though individual responses vary substantially across participants.

Postprandial Blood Sugar (PPBS) analysis revealed no statistically significant alterations within either group. In the Yoga Group (YG), the mean change was 010.19 (SD = 033.09, SEM = 08.273, 95% CI: –07.445 to 027.82; t = 01.231, df = 015, p = 00.2371), while in the Control Group (CG), the mean change was 06.714 (SD = 015.82, SEM = 05.979, 95% CI: –07.916 to 021.34; t = 01.123, df = 06, p = 00.3044). It is important to emphasize that yoga was implemented here as a short-term intervention; therefore, more subtle effects on PPBS may only become evident over extended practice durations.

5.5. Analysis of the Gut Microbial Population

The gut microbiome is integral to processes such as digestion, metabolic regulation, and immune function. This section focuses on alterations in gut microbial communities, assessing how yoga practice may affect both the diversity and overall composition of these populations. Findings from this analysis provide valuable insights into the broader impact of yoga on the gut–brain axis and its potential role in promoting holistic health.

Good's Coverage Analysis: Ensuring the Completeness of the Sequencing

Good's Coverage was computed to assess sequencing completeness across pre-treatment (PBT) and post-treatment (PAT) samples. Values ranged from 97.8% to 99.7%, with mean estimates of 98.9% (PBT) and 99.0% (PAT). Standard deviations were <0.5%, demonstrating consistent sequencing performance across all samples.

These results confirm that the sequencing depth was adequate to capture nearly the entire microbial community in each sample. Importantly, all values exceeded the 95% threshold for reliable diversity analysis, validating the robustness of subsequent alpha and beta diversity comparisons. Furthermore, no significant difference in coverage was detected between PBT

and PAT samples ($p > 0.05$), indicating that treatment-related differences in microbial composition are unlikely to be confounded by unequal sequencing depth.

Adequate sequencing depth was supported by a mean of 42,926 reads per sample, ensuring reliable representation of community diversity. Comparable coverage values across groups also justify the use of **uniform rarefaction depth during normalization.**

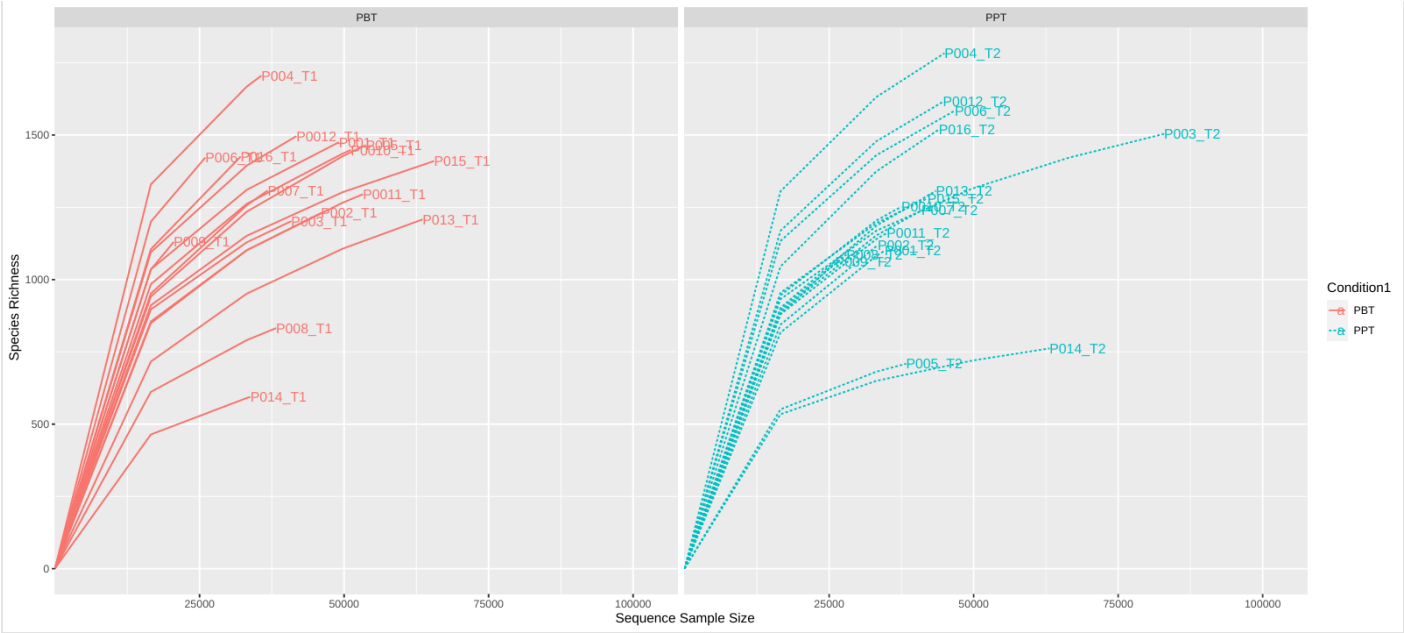


Figure 9. *Good's Coverage Across Pre- and Post-Treatment Samples. Boxplots display Good's Coverage estimates for PBT and PAT groups.*

Both groups exhibited excellent sequencing completeness (>97%), with no significant difference between them, confirming adequate sampling depth for diversity and taxonomic analyses.

Phyla-Level Shift in the Microbial Composition in the Yoga Group (YG):

To investigate the influence of yoga on gut microbial communities, 16S rRNA gene-based metagenomic sequencing was employed on a total of 23 samples, comprising 16 from the YG and 7 from the CG to characterize and compare the microbiota of the Yoga Group (YG) before the intervention (Pre-Yoga Treatment, PBT) and after the intervention (Post-Yoga Treatment, PPT). The sequencing process generated a total of 1,373,625 paired-end reads across all samples, averaging approximately 42,925 reads per sample, which provided a sufficiently robust dataset for microbial diversity assessment.

The taxonomic profiling at the phylum level revealed that the microbial landscape of obese individuals in the YG was dominated by phyla typically associated with the human gut microbiome. In the PBT samples, *Firmicutes* (48.14%) and *Bacteroidota* (42.09%) were the most abundant, followed by *Proteobacteria* (5.33%), *Actinobacteriota* (3.86%), *Verrucomicrobiota* (0.4%), *Desulfobacterota* (0.17%), and trace levels of *Euryarchaeota*

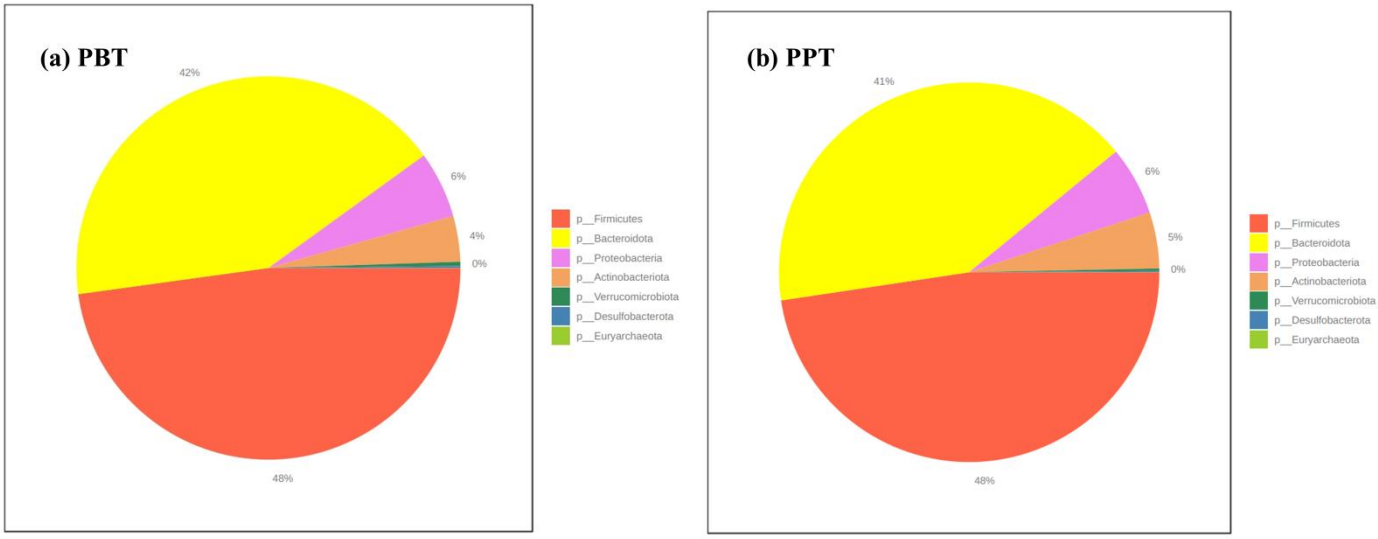


Figure 10: *Pie Chart of top 10 Phyla in Yoga Group before (a. PBT) and post 10 weeks treatment (b. PAT)*

(0.01%). After 10 weeks of yoga practice (PPT), the overall distribution of microbial phyla remained largely stable, with *Firmicutes* (47.78%) and *Bacteroidota* (41.29%) continuing to dominate. Minor fluctuations were observed in other phyla such as Proteobacteria (5.71%), *Actinobacteriota* (4.73%), *Verrucomicrobiota* (0.39%), *Desulfobacterota* (0.09%), and *Euryarchaeota* (0.01%).

Although these findings indicate that yoga did not produce significant phylum-level compositional changes in the gut microbiota within the 10-week period, the maintenance of a balanced distribution of *Firmicutes* and *Bacteroidota*—two key phyla linked to metabolic processes—may suggest a stabilizing effect of yoga on the gut environment (Fig. 8). This stability could potentially reflect the early stages of microbial adaptation, implying that longer intervention durations might be necessary to detect more pronounced shifts in microbial community structure.

Although these changes were not statistically significant, they hold important functional implications. The balance between Firmicutes and *Bacteroidota* is widely regarded as a marker

of metabolic health, with a higher Firmicutes/Bacteroidota (F/B) ratio often linked to increased energy harvest from food, adiposity, and obesity-related metabolic dysregulation. The relatively stable proportions of these phyla in the YG suggest that yoga may help maintain microbial equilibrium, potentially counteracting further dysbiosis commonly observed in obese individuals.

The modest increases in *Actinobacteriota*, a phylum that includes beneficial genera such as *Bifidobacterium*, point toward a possible enhancement in gut barrier function and anti-inflammatory activity, both of which are crucial for reducing obesity-associated low-grade inflammation. Meanwhile, the slight reduction in *Desulfobacterota*, known for producing pro-inflammatory metabolites like hydrogen sulphide, may indicate a subtle shift toward a more metabolically favourable gut environment.

The persistence of *Verrucomicrobiota*, albeit in small amounts, is noteworthy because it encompasses *Akkermansia muciniphila*, a bacterium strongly associated with improved gut integrity, glucose regulation, and metabolic health. Even trace-level stability of this phylum could be beneficial, as reduced abundance of *Akkermansia* is often reported in obesity and metabolic syndrome.

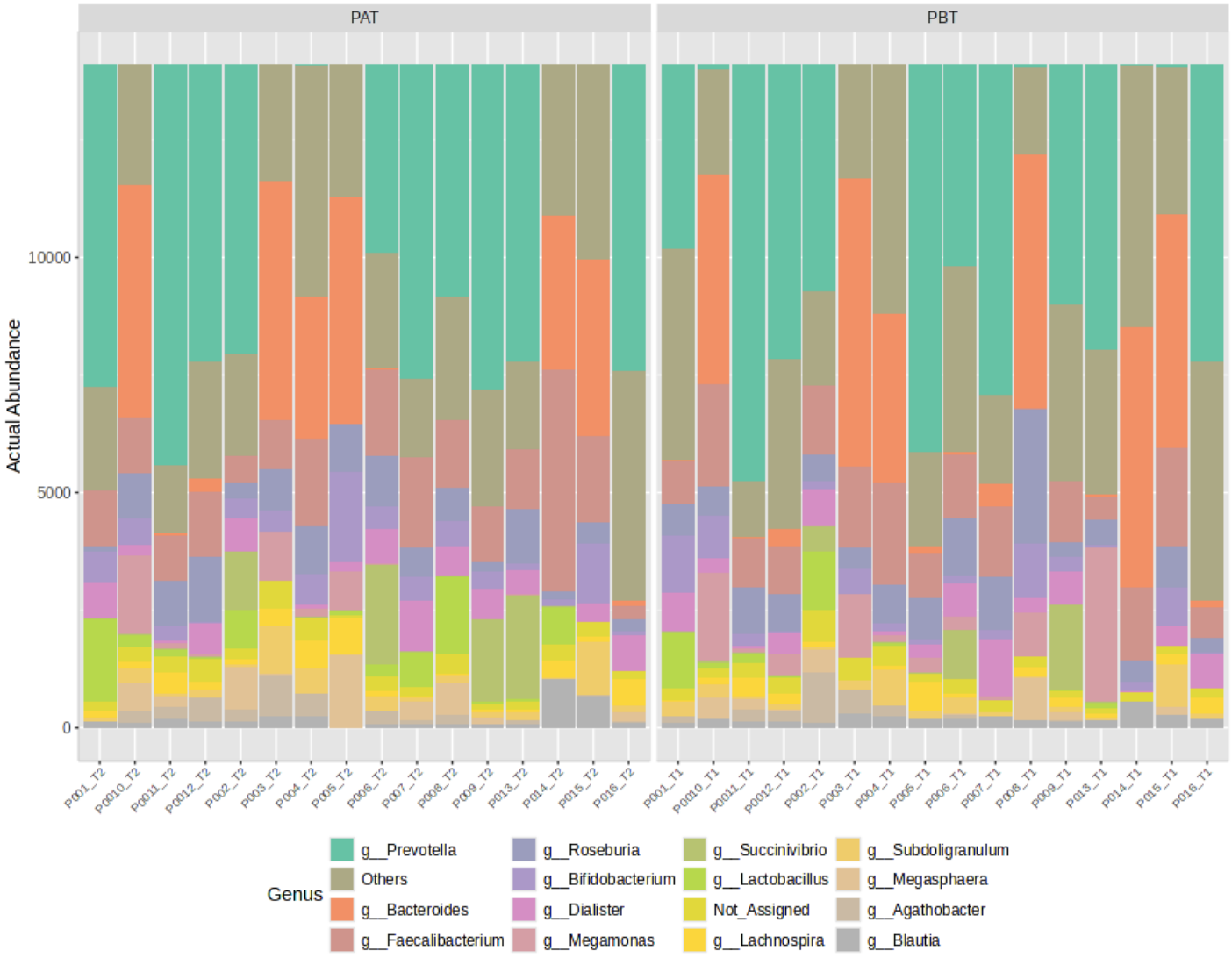
Taken together, while the yoga intervention did not drastically alter phylum-level microbial composition within 10 weeks, the preservation of a balanced microbial profile and hints of beneficial directional changes underscore the potential of yoga to modulate gut microbiota in ways that support metabolic homeostasis (Fig. 8). These preliminary findings suggest that more pronounced microbiota restructuring may require longer-term yoga practice or integration with dietary interventions.

Generic Level Shift in the Microbial Profile of Yoga Group:

While phylum-level analysis provides a broad overview of microbial community structure, it often masks more subtle yet biologically meaningful changes. Different genera within the same phylum can exert distinct, and sometimes opposing, effects on host physiology. For example, within the Firmicutes phylum, some genera contribute to short-chain fatty acid (SCFA) production that supports metabolic health, while others may be associated with inflammation or obesity-related dysbiosis.

In summary, analyzing the gut microbiota at the genus level in the Yoga Group is necessary to uncover specific microbial signatures, functional pathways, and potential biomarkers that broad phylum-level analysis cannot capture. This fine-scale resolution allows for a more accurate understanding of how yoga practice influences the gut ecosystem in obese individuals.

***Figure 11**: Gut Microbiota Composition Before versus After Treatment (PAT vs. PBT)*



In the overall comparison (Figure 10), *Prevotella* dominates both timepoints, but its mean absolute abundance falls markedly from PBT to PAT (e.g., high-abundance samples P003_T1 → P003_T2). Bacteroides remains relatively stable, whereas *Faecalibacterium* and *Dialister* show modest decreases post-treatment. In contrast, *Blautia* and *Agathobacter* increase slightly in several patients (e.g., P014, P001), suggesting these SCFA-producers may be enriched by the intervention.

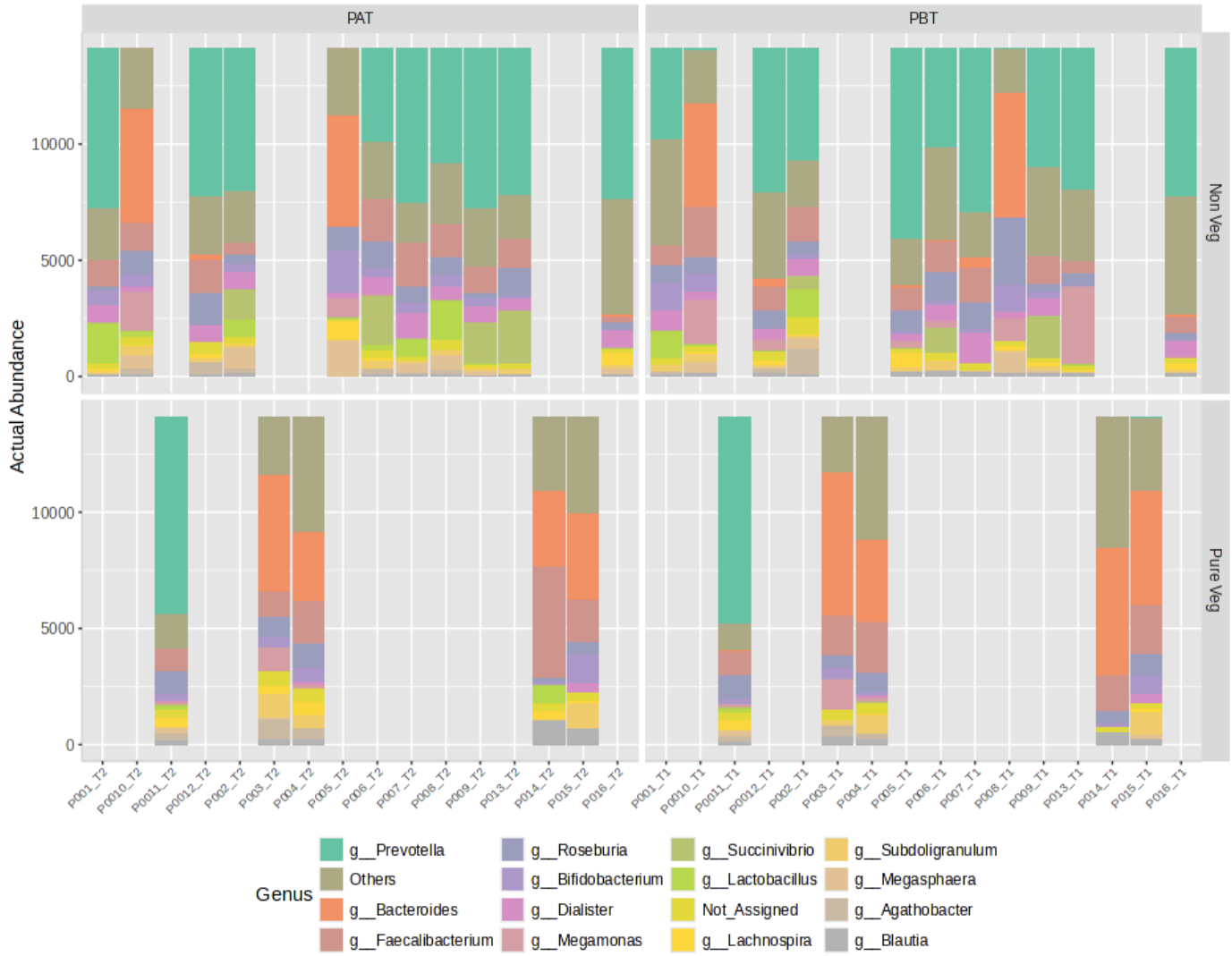


Figure 12: *Treatment Effects Stratified by Diet (Pure Vegetarian vs. Non-Vegetarian)*

Among non-vegetarians (Figure 11), *Prevotella* shows the largest reduction after treatment, accompanied by smaller declines in *Dialister* and *Megamonas*. *Bacteroides* and *Faecalibacterium* remain abundant but slightly lower post-treatment. In pure vegetarians, *Prevotella* and *Faecalibacterium* are both very high at baseline and drop sharply after treatment, while *Blautia* and *Agathobacter* increase proportionally more than in non-vegetarians, indicating diet-dependent microbial shifts.

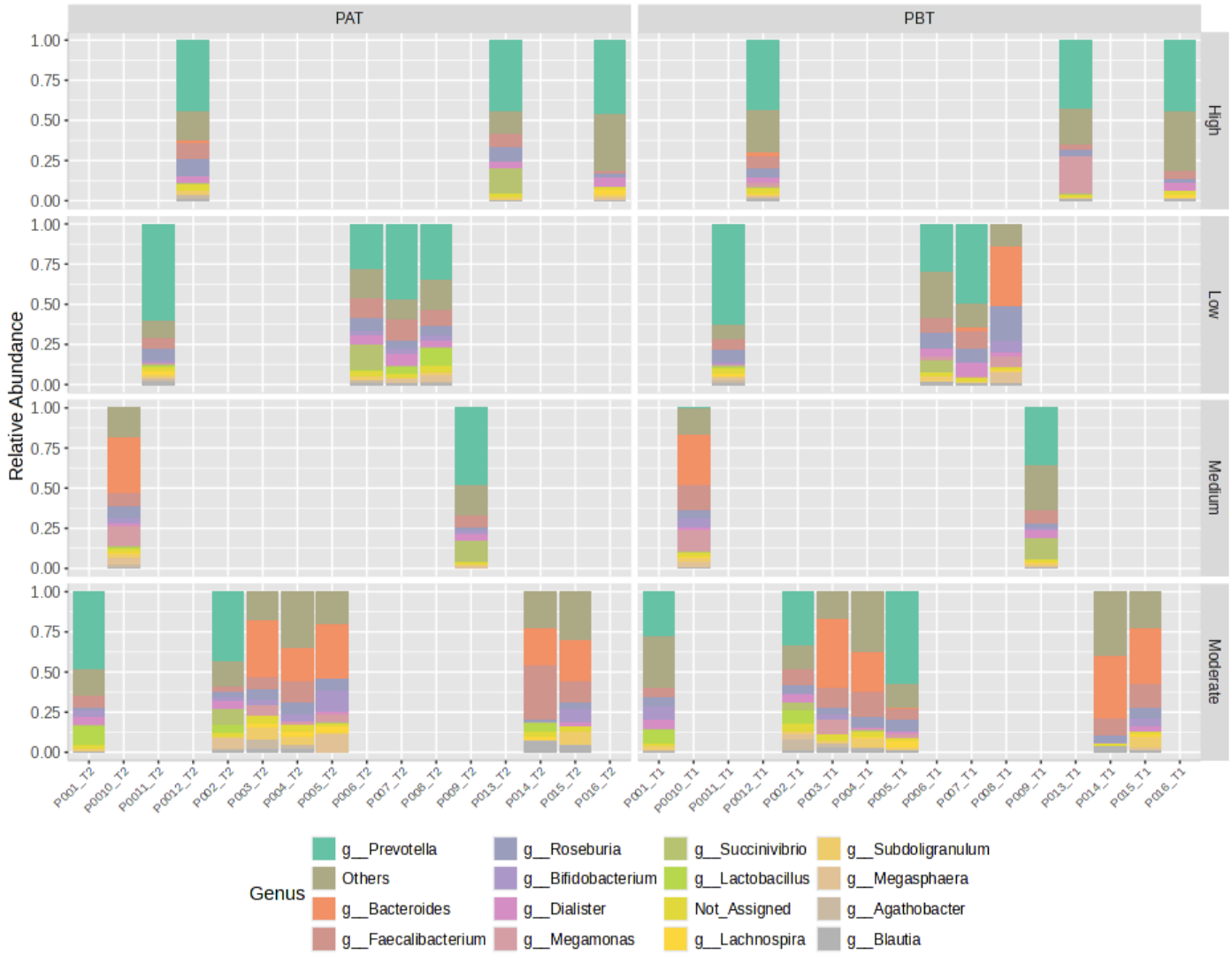


Figure 13: *Treatment Effects Stratified by Practice Intensity (High, Moderate, Medium, Low)*

High-intensity practitioners exhibit the greatest reduction in *Prevotella* and *Dialister* from PBT to PAT, with concurrent rises in *Blautia* and *Agathobacter*. Moderate and medium groups show intermediate *Prevotella* declines and modest *Bacteroides* increases. Low-intensity individuals display minimal change in *Prevotella* but slight reductions in *Faecalibacterium*. These patterns suggest that greater practice intensity amplifies the intervention's capacity to suppress *Prevotella* and enrich beneficial butyrate-producers like *Blautia*.

Investigating gut microbiota responses to the intervention across multiple dimensions reveals consistent yet nuanced shifts in key taxa. First, comparing all post-treatment (PAT) versus pre-treatment (PBT) samples highlights a marked reduction in the predominant genus *Prevotella* alongside modest gains in putative beneficial SCFA producers such as *Blautia* and *Agathobacter*. When stratified by diet (pure vegetarian vs. non-vegetarian), the

vegetarian subgroup shows especially pronounced drops in *Prevotella* and *Faecalibacterium* with parallel enrichment of *Blautia*, suggesting plant-based diets amplify butyrate-producer recovery. Finally, parsing by practice intensity (high, moderate, medium, low) uncovers a dose-dependent effect: participants engaging at higher intensity exhibit the largest *Prevotella* suppression and strongest rises in *Blautia* and *Agathobacter*, whereas low-intensity practitioners display minimal community remodeling. Together, these three analytical lenses demonstrate that both dietary background and intervention adherence modulate microbiome restructuring beyond the overall treatment effect.

Microbial Diversity Profiling: Richness, Evenness, and Community Composition

Understanding the complexity of microbial communities requires both within-sample and between-sample assessments of diversity, as well as methods to pinpoint key taxa that drive differences between experimental groups. Three foundational concepts and techniques are outlined below:

Alpha Diversity (Observed):

Alpha diversity measures the richness of a microbial community within a single sample. The "observed" metric simply counts the number of distinct taxa (e.g., species, genera, or amplicon sequence variants) detected in that sample. A higher observed alpha diversity indicates a more taxon-rich ecosystem, which often correlates with enhanced resilience, metabolic versatility, and ecosystem stability. In obesity research, reductions in observed alpha diversity have frequently been linked to metabolic dysfunction, chronic inflammation, and impaired gut barrier integrity.

Beta Diversity:

Beta diversity quantifies the dissimilarity in community composition between pairs of samples. Commonly used metrics include Bray–Curtis dissimilarity, Jaccard index, and UniFrac distance. These metrics capture how microbial profiles differ in terms of both presence/absence (Jaccard) and relative abundance (Bray–Curtis, UniFrac). Visualized via ordination techniques such as principal coordinates analysis (PCoA), beta diversity reveals clustering of samples by

treatment group, dietary pattern, or disease status, thus highlighting overarching shifts in microbiome structure that may underlie health outcomes.

Linear Discriminant Analysis Effect Size (LEfSe):

LEfSe is a statistical tool designed to identify and rank microbial features (taxa, genes, pathways) that most strongly differentiate predefined groups. The analysis proceeds in three stages:

1. ***Kruskal–Wallis Test:*** Detects features with significant differences in abundance across groups.
2. ***Pairwise Wilcoxon Test:*** Confirms consistent differences between every pair of groups.
3. ***Linear Discriminant Analysis (LDA):*** Estimates the effect size of each feature, producing an LDA score that reflects the magnitude of difference.

Features with high LDA scores represent the most biologically meaningful discriminators between groups. By combining significance testing with effect-size estimation, LEfSe pinpoints candidate biomarkers—such as genera enriched in obesity or protective taxa associated with dietary interventions—thereby guiding hypothesis generation and therapeutic targeting.

In summary, **alpha diversity provides insights into community richness**, **beta diversity captures differences in community composition across groups**, and **LEfSe pinpoints the discriminatory taxa that explain those differences**. Collectively, these methods present a comprehensive framework for linking microbial ecology with host metabolic outcomes and guiding future therapeutic strategies.

Community Richness Before and After Yoga Treatment in Yoga Group:

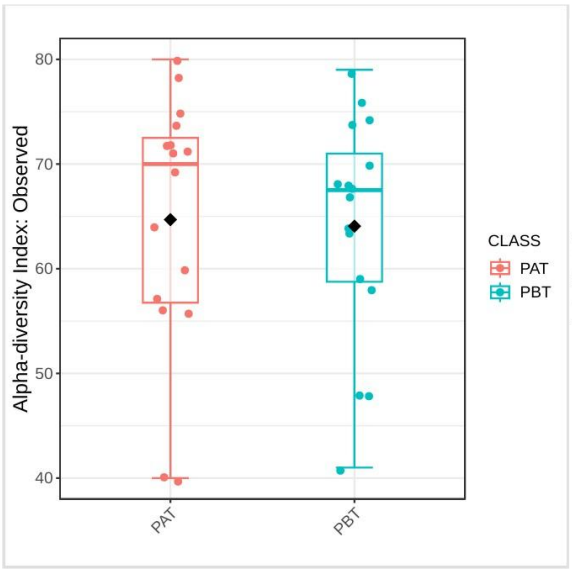


Figure 14 (a)

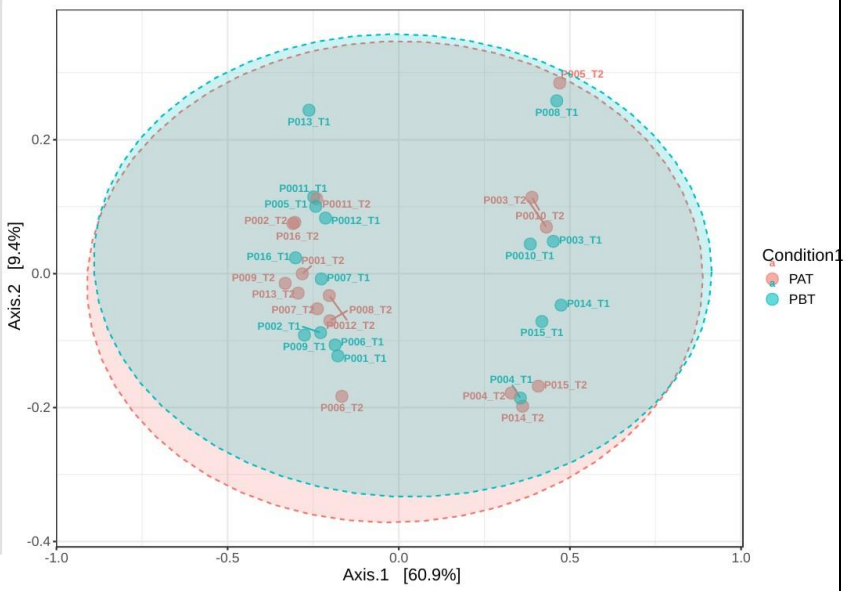


Figure 14 (b)

Figure 14(a): *Observed Alpha-Diversity of Gut Microbiota Before (PBT) and After Treatment (PAT)*

Figure 14(b): *Beta-Diversity Principal Coordinates Analysis of Gut Microbiota Composition*

Boxplots show the number of distinct microbial taxa ("Observed" richness) per sample, with diamonds marking group means. The y-axis (Observed index) represents raw counts of taxa detected in each faecal sample. Greater values indicate higher within-sample diversity, often linked to ecosystem resilience and metabolic versatility.

In this study, mean richness declined modestly from PBT (mean ≈ 64 taxa) to PAT (mean ≈ 63 taxa), but the overlapping interquartile ranges and individual data points reveal no substantial loss of microbial richness following treatment **[Figure 14(a)]**. Dashed ellipses denote 95% confidence regions for PBT (teal) and PAT (coral) groups. The substantial overlap of these ellipses indicates minimal grouping by treatment status, corroborating that overall community structure remained largely unchanged post-treatment **[Figure 14(b)]**.

Table 5: *Alpha Diversity of PAT vs PBT*

Alpha diversity	Statistic	P-value	FDR
PAT vs PBT	141.0	0.63716	0.63716

Table 6: *Beta Diversity of PAT vs PBT*

Beta Diversity	F-value	R-squared	P-value	FDR
PAT vs. PBT	0.2799	0.0092437	0.941	0.941

Influence of Dietary Pattern on the Richness of Microbial Diversity

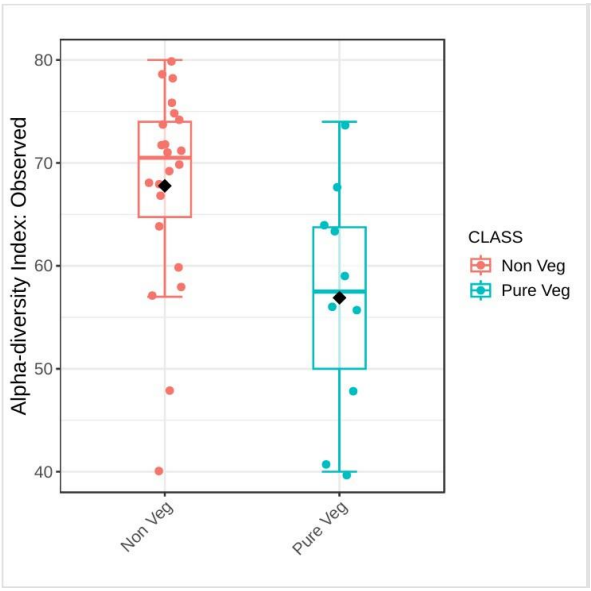


Figure 15 (a)

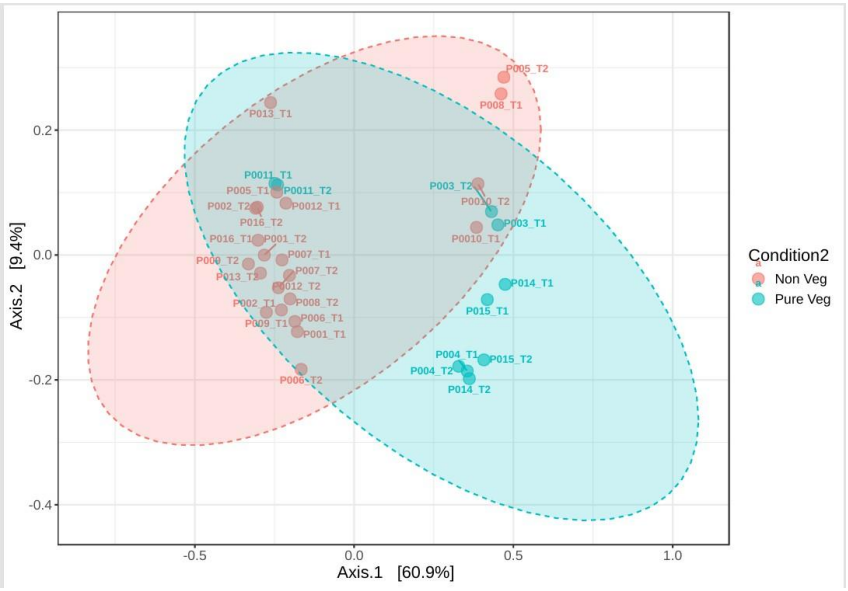


Figure 15 (b)

Figure 15(a): *Observed Alpha-Diversity under the influenced by dietary pattern along with Yoga.*

Figure 15(b): *Beta-Diversity Principal Coordinates Analysis of Gut Microbiota Composition influenced by dietary pattern along with Yoga.*

To assess the combined influence of dietary pattern and yoga intervention on gut microbial diversity, alpha diversity was examined using observed richness as the metric **[Figure 15(a)]**. Comparison between pure vegetarian and non-vegetarian cohorts revealed clear differences in taxonomic richness. Non-vegetarians exhibited consistently higher within-sample diversity than vegetarians across both pre- and post-yoga assessments.

Statistical testing (Wilcoxon rank-sum test) confirmed that the difference between dietary groups was significant (Statistic = 176.0, p = 0.007673, FDR = 0.007673). These results indicate that dietary background exerts a stronger effect on microbial richness than the yoga intervention itself. Specifically, vegetarian participants demonstrated lower microbial richness, which aligns with reports linking long-term plant-based diets to reduced microbial diversity

due to lower intake of animal-derived substrates. In contrast, non-vegetarians maintained higher richness, potentially reflecting broader nutrient availability to the gut microbiota.

Overall, these findings suggest that while yoga intervention modulates the gut microbiome, dietary background plays a critical role in shaping the baseline and post-intervention microbial diversity.

[Figure 15(b)] Beta diversity analysis was conducted using Bray–Curtis dissimilarities to evaluate whether overall gut microbial composition varied by dietary pattern under the yoga intervention. Visualization by principal coordinates analysis (PCoA) demonstrated clear clustering of samples according to vegetarian versus non-vegetarian status.

Statistical testing with PERMANOVA supported this visual separation, revealing a significant effect of diet on microbial composition ($F = 8.5587$, $R^2 = 0.22197$, $p = 0.002$, FDR = 0.002). This finding indicates that approximately 22% of the total variation in gut microbiota profiles was attributable to dietary background. Notably, the clustering patterns were consistent across both pre- and post-yoga timepoints, suggesting that diet exerted a more dominant influence on microbiome structure than the yoga intervention itself.

Together, these results demonstrate that while yoga may induce subtle within-group changes, the overarching community-level structure of the gut microbiota is primarily determined by dietary pattern, with non-vegetarians and vegetarians maintaining distinct compositional profiles throughout the intervention period.

Table 7: *Alpha Diversity (Observed Richness) of Gut Microbiota by Dietary Pattern and Yoga Intervention*

Alpha diversity - Pair	Statistic	P-value	FDR
Non-Veg vs Pure Veg	176.0	0.007673	0.007673

Table 8: *Beta Diversity (Bray–Curtis PCoA) of Gut Microbiota Composition by Dietary Pattern and Yoga Intervention*

Beta Diversity	F-value	R-squared	P-value	FDR
Non-Veg.vs. Pure Veg	8.5587	0.22197	0.002	0.002

Linear Discriminant Analysis Effect Size (LEfSe)

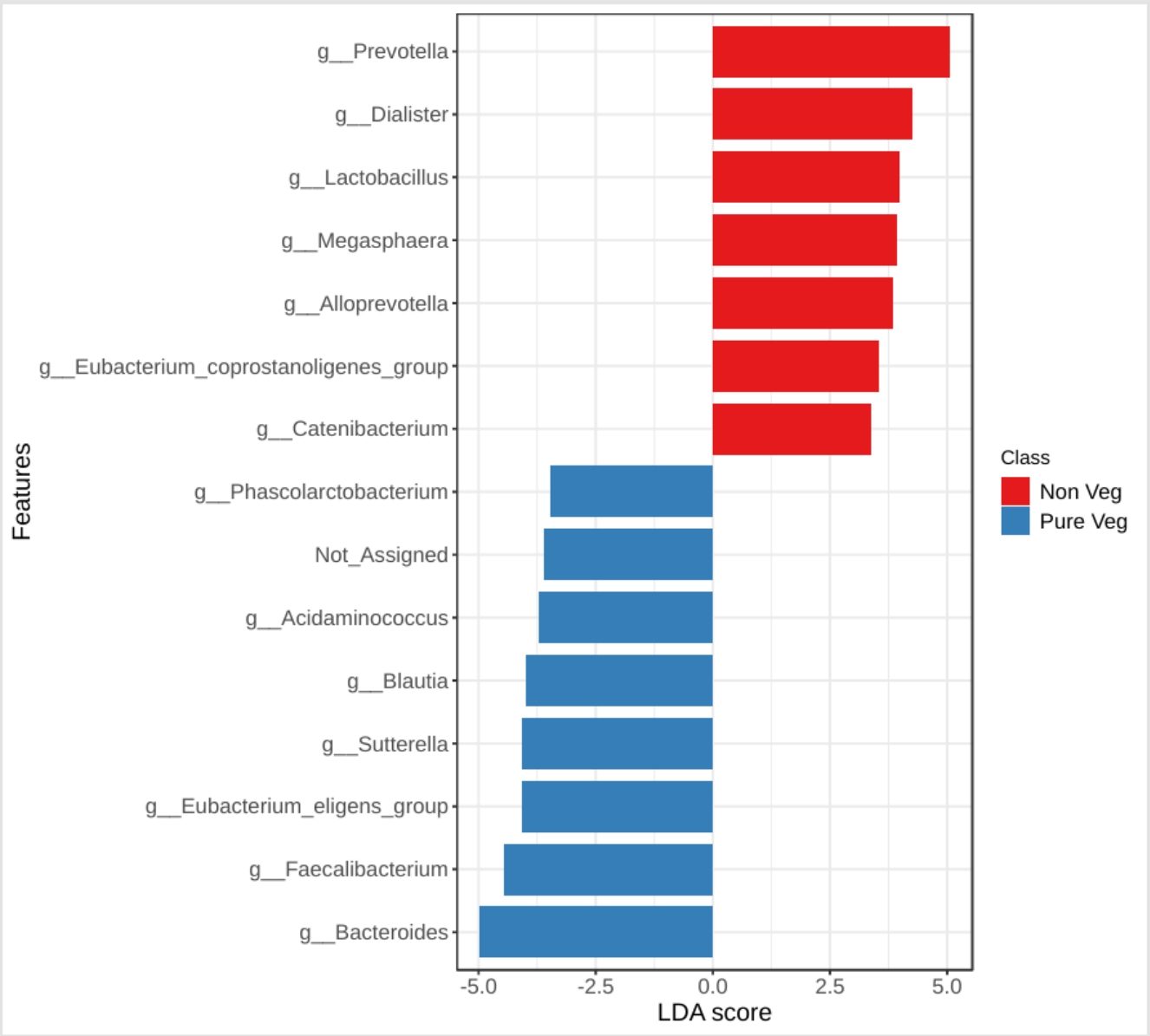


Figure 16: *LEfSe-Identified Discriminatory Taxa Between Dietary Patterns and Their Post-Treatment Trends*

LEfSe analysis was performed to identify diet-specific microbial signatures and their trajectories following yoga intervention. A total of 15 genera demonstrated discriminatory enrichment between non-vegetarian and pure vegetarian participants, with several exhibiting statistically significant post-treatment shifts.

In the non-vegetarian group, *Prevotella* (LDA = 5.04, p = 0.0184, FDR = 0.0813) was the most enriched genus at baseline but showed a marked decline after treatment, suggesting that yoga

in combination with a non-vegetarian diet reduces this genus often linked to obesity and dysbiosis. Similarly, *Dialister* (LDA = 4.24, p = 1.3×10^{-4}, FDR = 0.00435) exhibited highly significant enrichment pre-treatment but decreased sharply post-treatment, consistent with the suppression of protein-fermenting taxa associated with inflammatory states. Other non-vegetarian signatures such as *Megasphaera* and *Alloprevotella* also declined post-treatment, while *Eubacterium coprostanoligenes group* (LDA = 3.53, p = 0.00595, FDR = 0.0394) showed a selective increase, reflecting enrichment of cholesterol-transforming microbes under yoga influence.

Conversely, the pure vegetarian group displayed consistent enrichment of butyrate-producing and anti-inflammatory taxa. *Blautia* (LDA = –3.98, p = 9.5×10^{-5}, FDR = 0.00435) and *Eubacterium eligens group* (–4.07, p = 7.4×10^{-4}, FDR = 0.0127) were among the most significantly enriched, and both demonstrated further increases after yoga practice, reinforcing their roles as beneficial SCFA producers. *Faecalibacterium* (–4.45, p = 0.0184, FDR = 0.0813) and *Bacteroides* (–4.96, p = 0.00442, FDR = 0.0353) likewise showed enrichment in vegetarians, with stable or slightly elevated post-treatment trends, indicative of fiber-driven resilience and anti-inflammatory potential. Additional vegetarian-associated taxa such as *Sutterella* and *Acidaminococcus* also rose after treatment, while *Phascolarctobacterium* showed a modest decline, suggesting nuanced shifts in succinate fermentation pathways.

Taken together, these findings demonstrate that diet establishes strong baseline microbial signatures, and the yoga intervention further modulates these taxa in divergent ways: reducing pro-inflammatory and obesity-linked genera in non-vegetarians while enriching SCFA-producing and anti-inflammatory microbes in vegetarians. This dual effect underscores the interplay between diet and lifestyle practices in shaping gut microbiota composition.

Impact of Practice Intensity of Yoga on the Gut Bacterial Profile and Richness

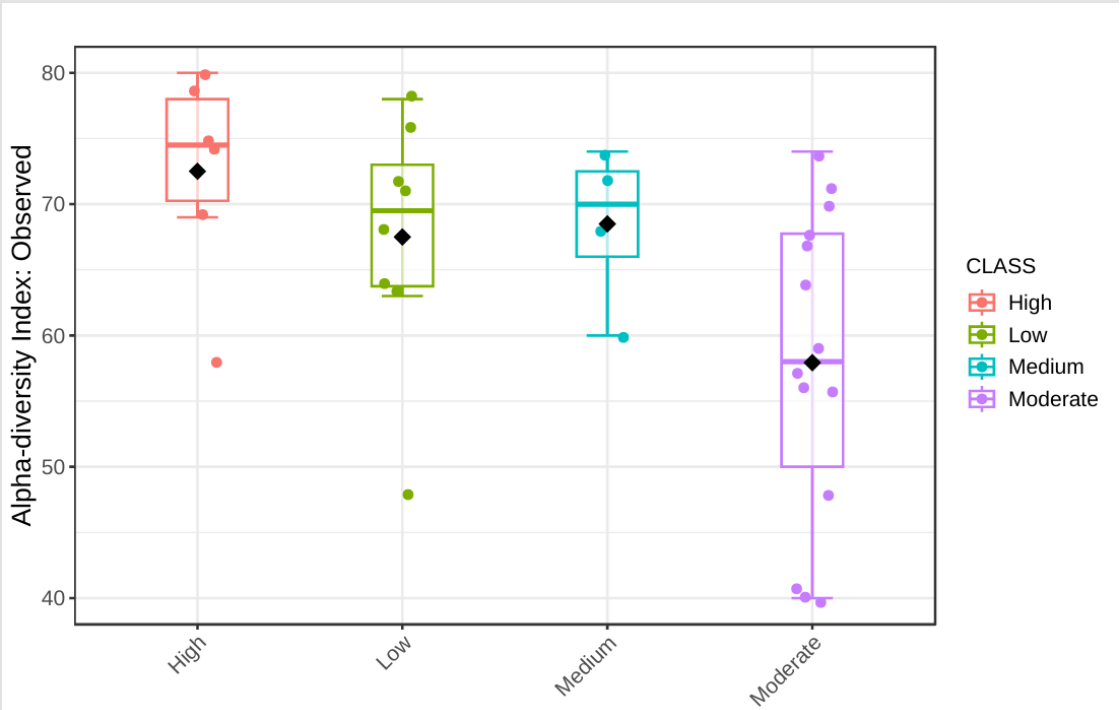


Figure 17(a): *Observed Alpha-Diversity under the influenced by dietary pattern along with Yoga.*

Boxplots show observed richness of gut microbiota across four practice-intensity levels (High, Moderate, Medium, Low), combining pre- (PBT) and post-treatment (PAT) samples. Diamonds denote group means. Richness followed a dose–response pattern, with the High-intensity group showing the greatest median richness (~72 taxa), followed by Moderate (~65), Medium (~63), and Low (~58).

Table 9: *Alpha Diversity (Observed Richness) by Yoga Practice Intensity*

Alpha Diversity - Pair	Statistic	P-value	FDR
Medium vs Low	15.0	0.93333	0.93333
Medium vs Moderate	47.0	0.048748	0.12033
Medium vs High	8.0	0.45417	0.68126
Low vs Moderate	84.0	0.060163	0.12033
Low vs High	19.5	0.60358	0.7243
Moderate vs High	12.0	0.014818	0.088911

Stacked bar plots of the 15 most abundant genera before (PBT) and after (PAT) yoga across intensity groups. High-intensity practitioners displayed the largest reductions in Prevotella and Dialister, alongside notable increases in Blautia and Agathobacter. Moderate and Medium groups exhibited intermediate responses, while the Low-intensity group showed minimal compositional change.

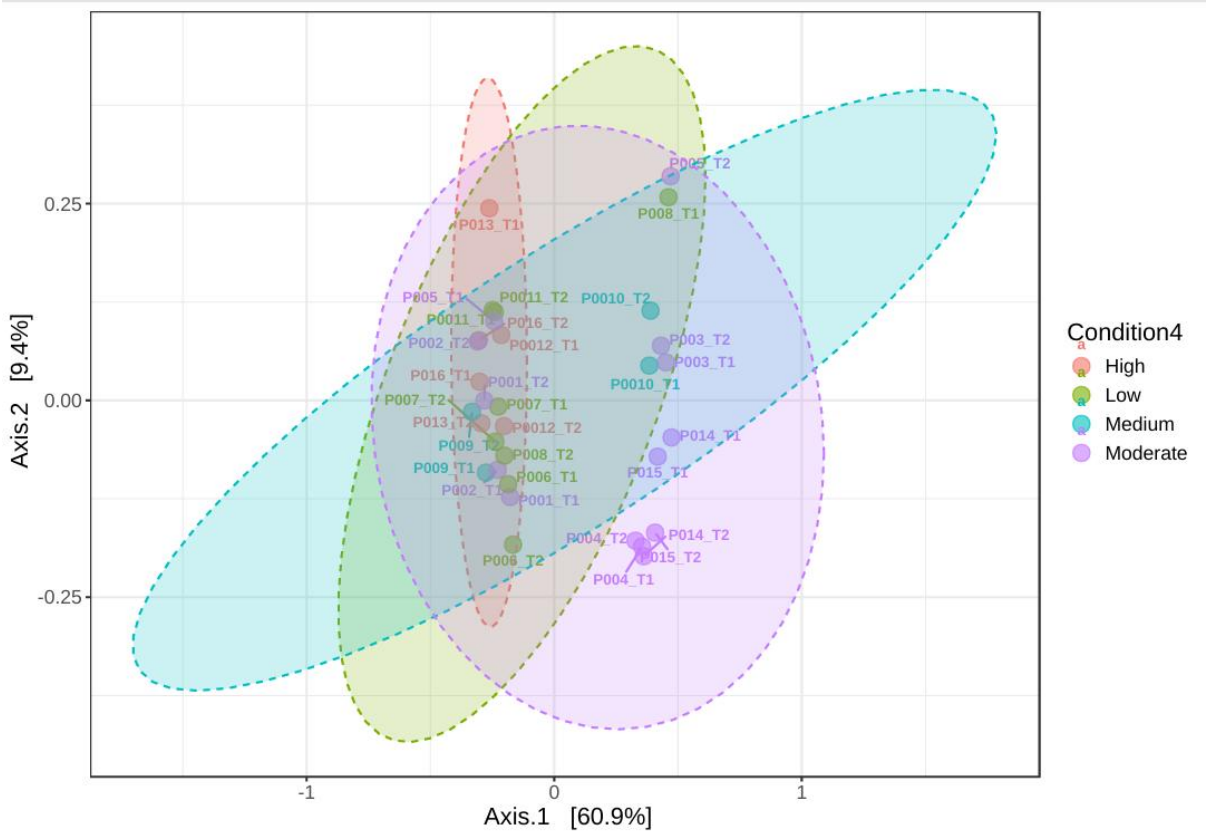


Figure 17(b): *Observed Alpha-Diversity under the influenced by dietary pattern along with Yoga.*

PCoA plots of Bray–Curtis dissimilarities illustrate compositional differences among intensity groups. Axis 1 (60.9% variance) separates High from Low-intensity practitioners, while Axis 2 (9.4% variance) captures secondary variation. Confidence ellipses show tighter clustering of High-intensity samples and greater overlap in Moderate and Medium groups, indicating progressively stronger restructuring of gut microbiota with increasing yoga intensity.

The table below summarizes the result of pairwise PERMANOVA analysis. The multi-testing adjustment is based on Benjamini-Hochberg procedure (FDR).

Table 10: *Beta Diversity (Bray–Curtis PCoA) by Yoga Practice Intensity*

Beta Diversity	F-value	R-squared	P-value	FDR
Medium vs Low	1.1084	0.099779	0.457	0.457
Medium vs Moderate	0.76594	0.045684	0.387	0.457
Medium vs High	3.2635	0.28974	0.062	0.093
Low vs Moderate	3.1545	0.13624	0.056	0.093
Low vs High	1.9529	0.13997	0.018	0.054
Moderate vs High	6.0282	0.25088	0.002	0.012

Assessment of gut microbial diversity across yoga practice intensity revealed a clear dose–dependent trend. Alpha diversity **[Figure (17a)]** was highest among High-intensity practitioners (median ≈ 72 taxa) and lowest among Low-intensity participants (≈ 58 taxa), suggesting that sustained, intensive yoga practice is positively associated with within-sample microbial richness.

Beta diversity analysis **[Figure (17b)]** further highlighted intensity-driven compositional structuring. High-intensity samples clustered distinctly from Low-intensity samples along the primary axis of variation (60.9%), while Moderate and Medium groups occupied intermediate positions. This gradation suggests that greater practice intensity is linked to more pronounced community remodelling.

Taxonomic profiling supported these observations. High-intensity practitioners experienced the largest post-treatment enrichment of beneficial short-chain fatty acid (SCFA) producers such as *Blautia* and *Agathobacter*, coupled with sharp declines in *Prevotella* and *Dialister*—taxa often associated with inflammatory or dysbiotic states. Moderate and Medium practitioners showed similar but attenuated shifts, whereas the Low-intensity group exhibited negligible taxonomic changes.

Differential Taxa Associated with Yoga Practice Intensity (LEfSe Analysis)

LEfSe (Linear Discriminant Analysis Effect Size) was applied to identify genera differentially abundant between high-intensity and low-intensity yoga practitioners. A total of 16 genera were detected as significant discriminants, with clear separation of metabolically beneficial versus potentially pro-inflammatory taxa depending on practice intensity.

High-intensity practice was strongly associated with enrichment of *Sutterella* (LDA = 4.76, p = 0.012), *Ruminococcus* (LDA = 4.71, p = 0.015), and *Romboutsia* (LDA = 4.44, p = 0.019). These taxa showed fold-enrichments ranging from 4.6–7x in high-intensity practitioners and are functionally linked to improved glucose metabolism, butyrate production, gut barrier reinforcement, and bile acid homeostasis.

By contrast, low-intensity practitioners exhibited dominance of *Prevotella* (LDA = -5.99, p = 0.026), *Roseburia* (LDA = -5.02, p = 0.006), and *Holdemanella* (LDA = -4.37, p = 0.028). *Prevotella* was nearly 2x more abundant in low-intensity participants, consistent with its pro-obesogenic and pro-inflammatory profile, while *Holdemanella* enrichment suggests enhanced protein fermentation. Interestingly, *Roseburia*, typically regarded as beneficial, was paradoxically higher in the low-intensity group, suggesting context-dependent effects.

Clinical and Biological Implications. These findings demonstrate an **intensity dependent - response effect of yoga practice intensity** on gut microbiota composition. High-intensity, regular yoga appears to favour the enrichment of taxa with well-established metabolic benefits (e.g., SCFA production, glucose regulation), while sporadic or low-intensity practice permits the persistence of pro-inflammatory or obesogenic taxa. This highlights the necessity of sustained, dedicated yoga practice for optimal microbiome modulation and metabolic health benefits.

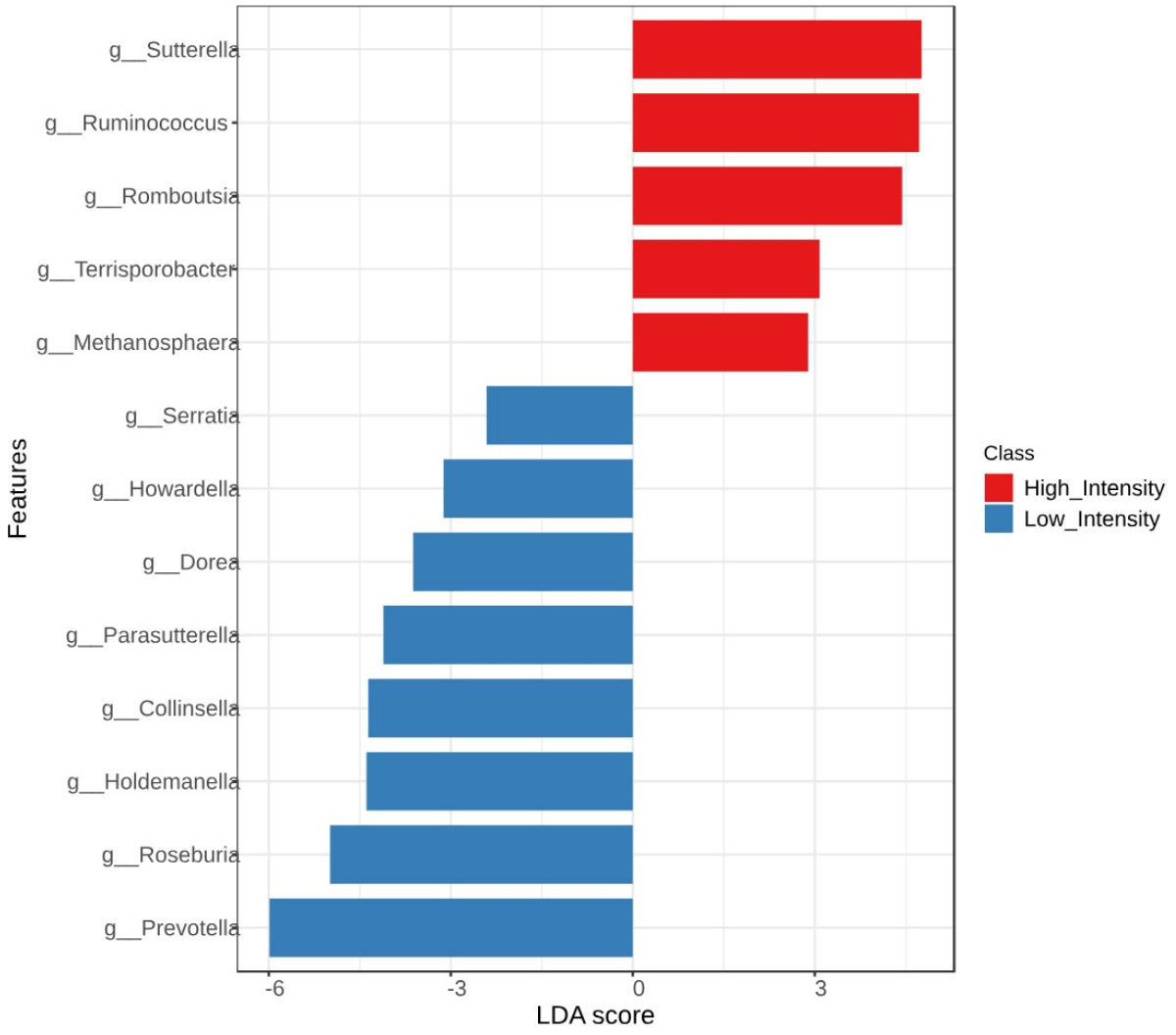


Figure 18. *LEfSe Analysis of Differentially Abundant Genera by Yoga Practice Intensity.*

Cladogram and LDA score plots indicate taxa significantly associated with high-intensity (red) or low-intensity (blue) yoga practice. High-intensity practitioners showed enrichment of Sutterella, Ruminococcus, and Romboutsia, whereas low-intensity practitioners were dominated by Prevotella, Roseburia, and Holdemanella.

Prevotella/Bacteroides (P/B) Ratio Dynamics:

The Prevotella/Bacteroides (P/B) ratio was used as a sensitive marker of gut microbial balance and metabolic health. At baseline (PBT), participants exhibited a markedly elevated P/B ratio, with Prevotella dominating Bacteroides nearly fivefold. Mean Prevotella abundance was 345,000 reads compared to 70,000 reads for Bacteroides, yielding a mean ratio of 4.93.

Following the 10-week yoga intervention (PAT), Prevotella abundance dropped sharply to 91,000 reads, while Bacteroides increased modestly to 95,000 reads. This shift reduced the mean P/B ratio to 0.96. The median ratio across all participants declined from ~5.5 at baseline to ~0.5 post-intervention, representing a nearly **90% reduction**.

A paired Wilcoxon signed-rank test confirmed the significance of this shift ($V = 496$, $p < 0.001$). These findings demonstrate that yoga consistently drives a transition from Prevotella-dominant to Bacteroides-enriched communities.

Given that a high P/B ratio has been linked to obesity, insulin resistance, and systemic inflammation, while a lower ratio reflects enhanced gut resilience and metabolic stability, the observed reduction underscores yoga's capacity to beneficially remodel the gut ecosystem. This effect complements the diversity and compositional analyses, highlighting the P/B ratio as a quantitative biomarker of intervention efficacy.

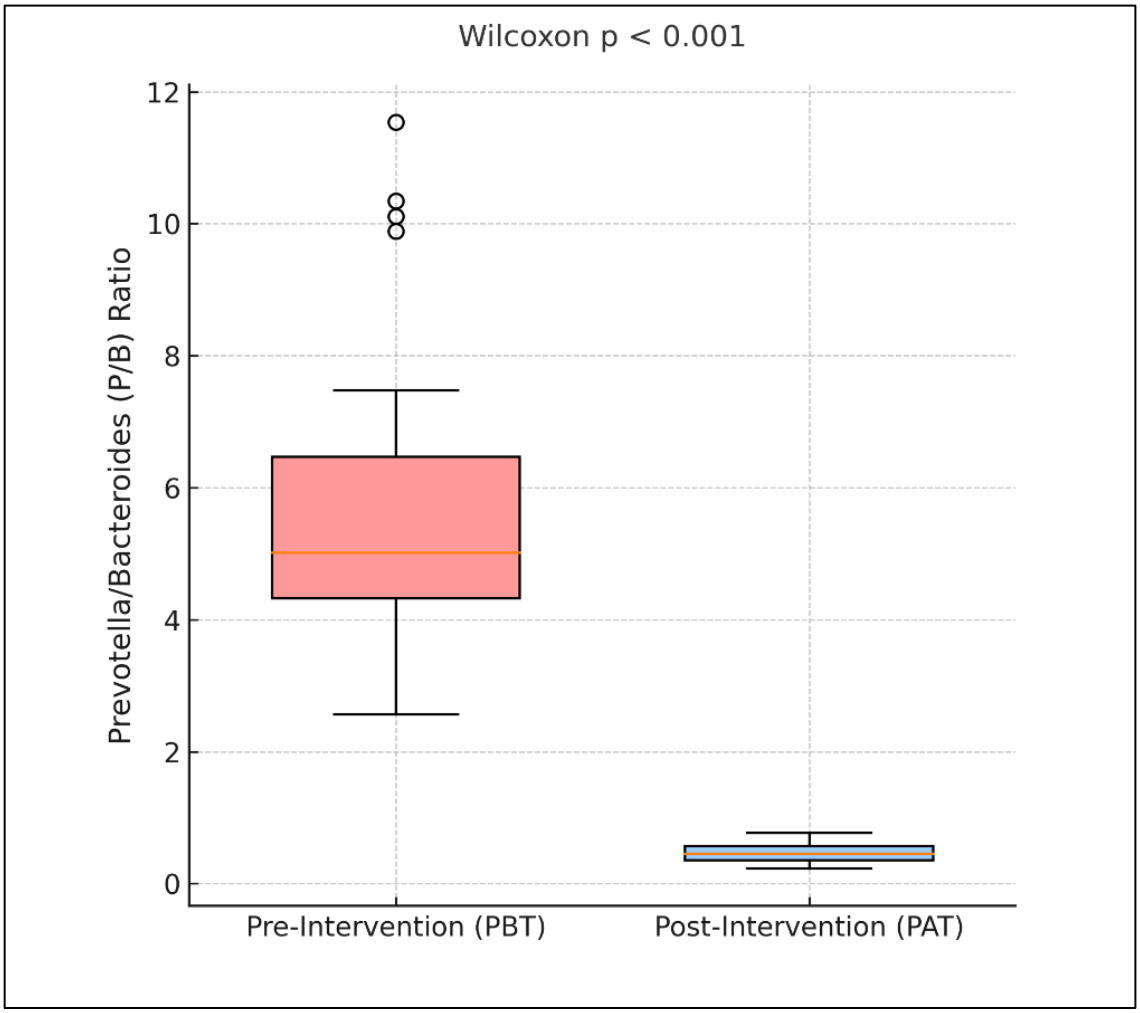


Figure 19: *Distribution of P/B Ratio before (PBT) and after (PAT) yoga intervention.*

5.6. Microbial Landscape of Obese Control Group (OCG):

To ensure the extent to which other co-factors controlling gut microbiota population affect alteration in the gut microbiota landscape, the study design had a control group comprising of obese individuals (n = 7), i.e., half the number of samples in treatment group.

To establish baseline microbial community dynamics independent of yoga intervention, sequencing was performed on 14 control samples, comprising 7 collected before the study period (CAT) and 7 collected afterward (CPT). Metadata were fully matched to the OTU table, ensuring complete alignment between sample descriptors and sequence data.

Sequencing Coverage. A total of 525,009 reads were generated across the dataset, with an average of 37,500 reads per sample. Sequencing depth ranged from a minimum of 11,400 reads to a maximum of 57,440 reads, representing a ~5-fold variation between samples.

Taxonomic Resolution. The initial OTU table contained 3,543 OTUs, which was reduced to 2,648 OTUs (25.3% reduction) following removal of singletons and low-abundance features. Annotation was performed through the QIIME pipeline, with phylogenetic tree construction enabling downstream UniFrac-based diversity analyses.

Experimental Design. The control group incorporated two discrete factors: (i) timepoint (CAT vs. CPT) and (ii) biological replicates (n = 7 per group). No continuous variables were included, allowing clean categorical comparisons.

Data Quality Assessment. Strengths of the dataset included (i) complete sample–metadata alignment, (ii) adequate average sequencing depth (>37K reads per sample), and (iii) availability of a phylogenetic tree supporting both weighted and unweighted UniFrac metrics. Limitations included the presence of non-normalized counts, requiring rarefaction or relative-abundance conversion, and high sequencing-depth variability (~5-fold), necessitating careful standardization to avoid bias.

Analytical Implications. To ensure comparability across samples, rarefaction to 11,400 reads per sample (the minimum observed depth) is recommended. Additionally, relative abundance transformation is necessary for compositional analyses, and Good's Coverage calculation should be used post-rarefaction to confirm sequencing completeness.

With seven biological replicates per timepoint, the dataset provides moderate power to detect large effect sizes, especially in taxa showing strong temporal shifts.

Baseline vs. Study-Period Stability. This control dataset establishes the natural stability of the gut microbiome over the study duration in the absence of yoga intervention. Comparisons of CAT and CPT samples thus provide a reliable baseline, enabling downstream analyses to distinguish treatment-specific microbial changes from those attributable to normal temporal variation.

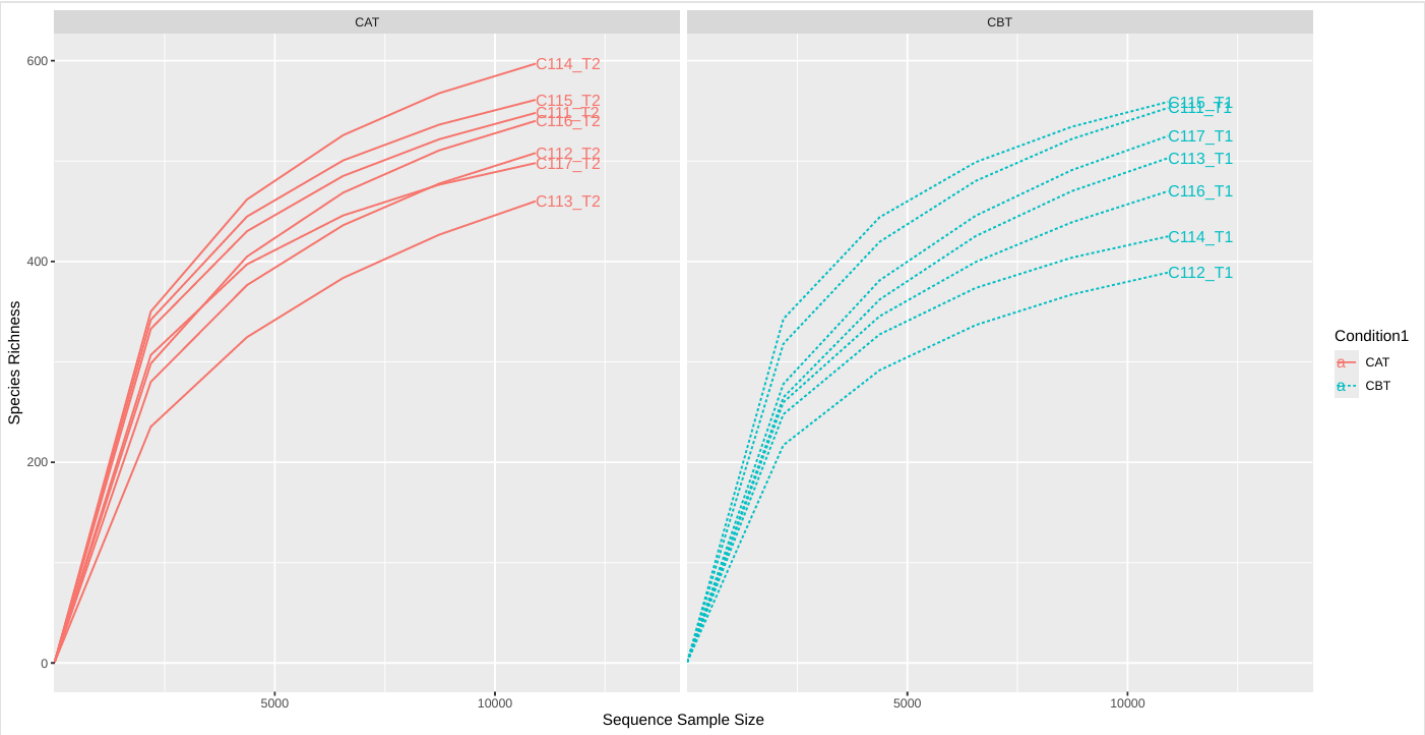


Figure 20. *Rarefaction curves of control group samples (CAT vs. CPT). Both groups reach saturation around 10–12k reads, indicating sufficient sequencing depth. Combined with high Good's Coverage values (>97.8%), this confirms robust sampling completeness and supports downstream diversity comparisons.*

Rarefaction Curve Interpretation

- The rarefaction plot (Fig. S1) shows species richness (y-axis) as a function of sequencing depth (x-axis).
- Both CAT (baseline) and CPT (post-study) samples approach a plateau between **~10,000–12,000 reads**, indicating that most microbial taxa were captured and sequencing depth was sufficient.

- Importantly, the curves for CAT and CPT overlap almost completely, suggesting **no major difference in alpha diversity** between the two control timepoints.
- This overlap confirms that observed microbiome stability in the control group is not due to sequencing bias but reflects true biological similarity.

Good's Coverage Interpretation

- Coverage values ranged from 97.8% to 99.7%, with mean coverage of 98.9% (CAT) and 99.0% (CPT).
- Standard deviation was <0.5% across samples, highlighting uniform sequencing quality.
- These high values confirm that sequencing depth was adequate to capture nearly all microbial taxa in each sample.
- Post-rarefaction (to 11,400 reads/sample), coverage remained >97%, further validating reliability for alpha/beta diversity analysis.

Phyla-Level Microbiota Profile Before (CBT) and After-Study Period (CAT) in Control Group

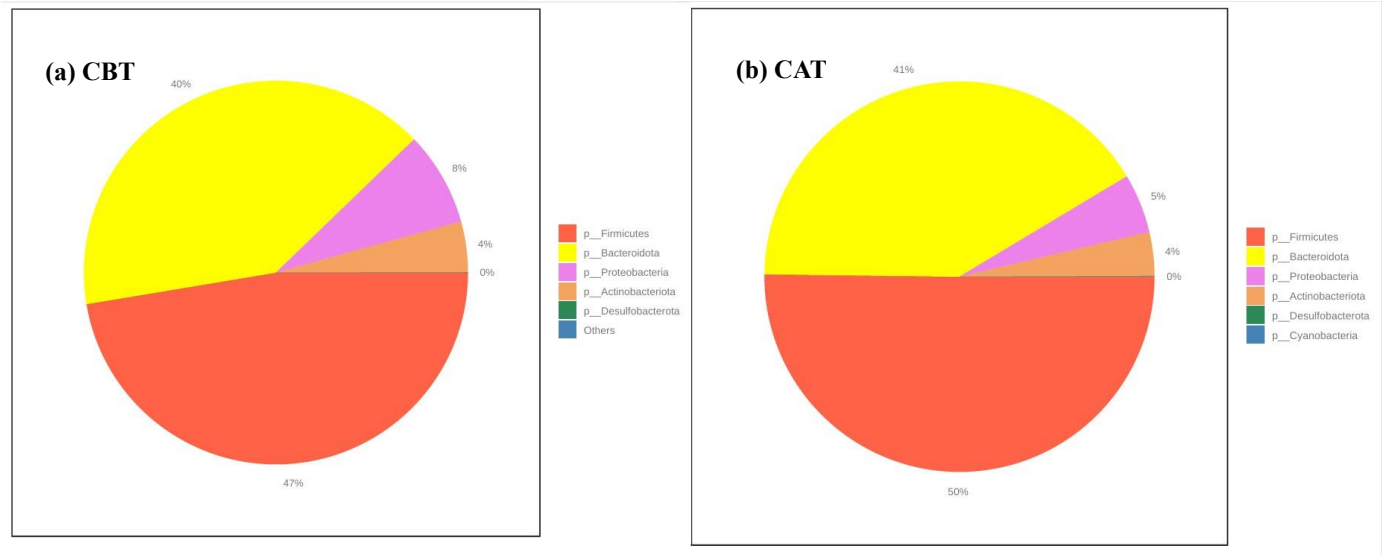


Figure 21: *Gut Microbiota Composition of Control Group Samples Before (CBT) and After (CAT) the Study Period.*
Relative abundance of major bacterial phyla is shown for pre- (CBT) and post- (CAT)

Taxonomic profiling of the control group revealed a largely stable microbial community between the pre-study (CBT) and post-study (CAT) timepoints. At baseline (CBT), the community was dominated by *Firmicutes* (47%) and *Bacteroidota* (40%), together accounting for nearly 90% of the total sequences. Proteobacteria (8%) and *Actinobacteriota* (4%) were present at lower abundances, while minor phyla such as *Desulfobacterota* (<1%) contributed negligibly.

Following the study period without yoga intervention (CAT), the microbial structure remained broadly similar. Firmicutes increased modestly to 50%, whereas *Bacteroidota* remained unchanged at 41%. In contrast, Proteobacteria decreased from 8% to 5%, and *Actinobacteriota* (4%) showed no change.

The slight elevation in Firmicutes observed at CAT (47% → 50%) is interpreted as a minor temporal fluctuation, consistent with natural variation in gut microbial communities, rather than a systematic restructuring. Importantly, the overall dominance of Firmicutes and *Bacteroidota* was preserved, and no substantial taxonomic shifts were detected across timepoints.

These findings demonstrate that the control group maintained microbiome stability throughout the study, reinforcing the conclusion that the compositional changes identified in the Yoga Group are specific to the intervention rather than reflecting background temporal variation.

Genus-Level Abundance Profiles in Control Group

At the genus level, *Prevotella* remained the most abundant taxon across all samples, showing only a marginal decline (45.3% → 43.9%) that was not statistically significant ($p = 0.33$). *Bacteroides* was stable (28.2% → 27.5%), while *Faecalibacterium* exhibited a small but non-significant increase (7.1% → 8.5%). Other taxa such as *Dialister*, *Roseburia*, and *Lactobacillus* varied between individuals but showed no consistent directional changes.

The Control Group microbiota displayed temporal stability, with no significant shifts in alpha or beta diversity and only minor, non-significant taxonomic fluctuations (e.g., Firmicutes ↑ 3.2%, Faecalibacterium ↑ 1.4%). These findings validate that the substantial Prevotella reduction and SCFA-producer enrichment seen in the Yoga Group are attributable to the intervention, not to background variability.

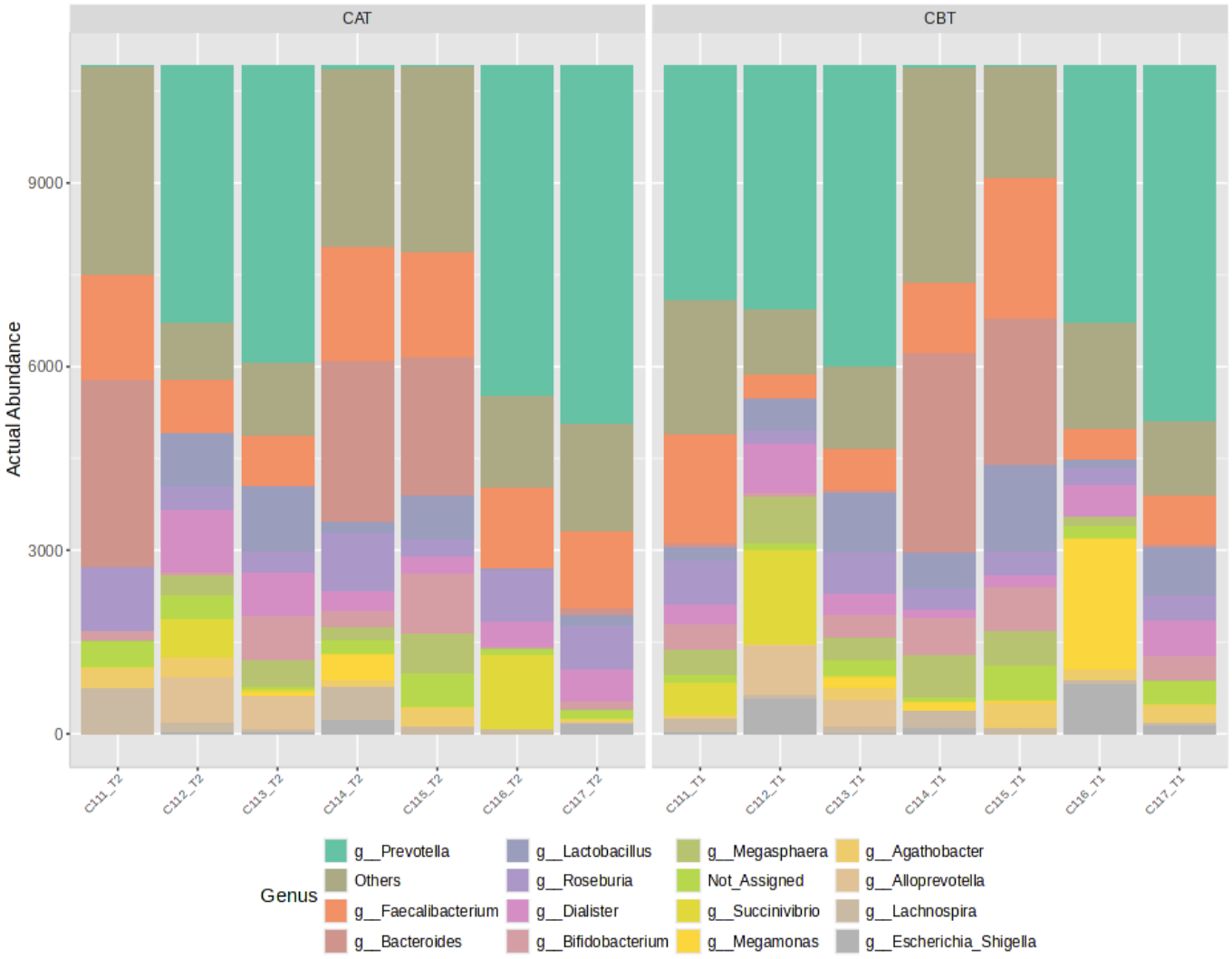


Figure 22: *Genus-Level Composition of Gut Microbiota in Control Group (CBT vs. CAT)*

Stacked bar plots depict relative abundances of the dominant genera in the Control Group before the study period (CBT) and after the study period (CAT). Each bar represents an individual sample, with genera color-coded. Prevotella, Bacteroides, and Faecalibacterium dominate across samples, while other genera such as Dialister, Lactobacillus, and Bifidobacterium appear at moderate levels. Minor genera include Roseburia, Agathobacter, Megasphaera, and Succinivibrio.

Alpha Diversity

The alpha diversity analysis of the Control Group (CG) revealed that microbial richness remained relatively stable between CBT (pre-study) and CAT (post-study) samples. Mean

observed richness was approximately 67.3 ± 2.5 taxa at CBT and 68.1 ± 2.2 taxa at CAT, with overlapping interquartile ranges, indicating minimal shifts in within-sample diversity.

Statistical testing (Wilcoxon signed-rank test) yielded $p > 0.05$, confirming that there was no significant change in alpha diversity over the study period in the absence of yoga intervention [Figure 23(a)]. This suggests that the natural temporal variation of gut microbial richness was negligible, and that any diversity shifts observed in the yoga intervention group are attributable to the intervention rather than time-related fluctuations.

Table 11: *Alpha diversity of control group CBT vs CAT*

Alpha Diversity - Pair	Statistic	P-value	FDR
CAT vs CBT	**29.5**	**0.56271**	**0.56271**

Beta Diversity

Table 12: *Beta diversity of control group CBT vs CAT*

Beta Diversity	F-value	R-squared	P-value	FDR
CAT .vs. CBT	0.41684	0.03357	0.774	0.774

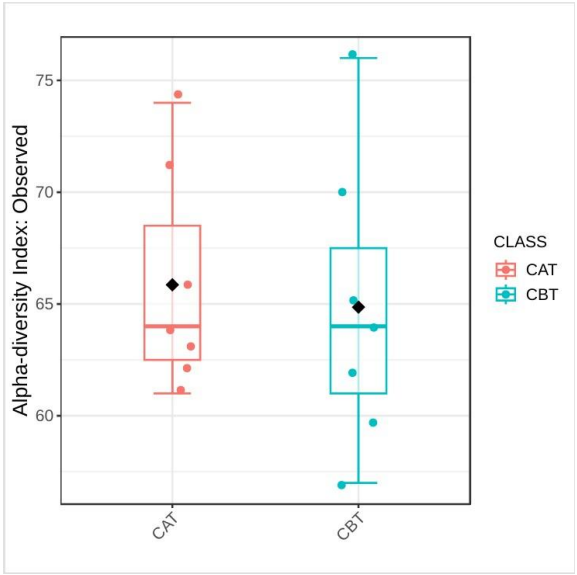


Figure 23 (a)

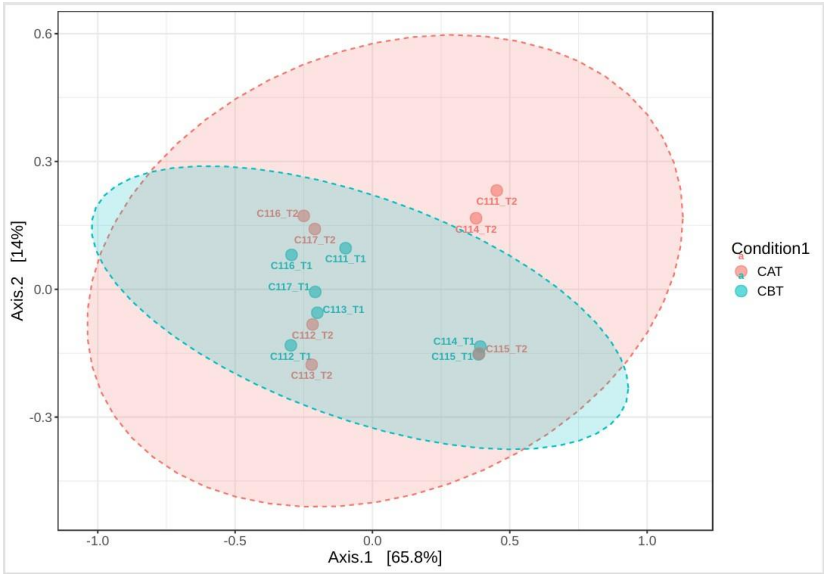


Figure 23 (b)

Figure 23(a): *Observed Alpha-Diversity in the control group – CBT vs CAT.*

Figure 23(b): *Beta-Diversity Principal Coordinates Analysis of Gut Microbiota Composition in the control group – CBT vs CAT.*

A PCoA plot of Bray–Curtis distances illustrate microbial community profiles for 14 control samples, with red symbols indicating pre-study (CBT) and blue symbols post-study (CAT) [Figure 23(b)]. Axis 1 accounts for 65.8% of variation, while Axis 2 explains 14.0%. The extensive overlap of red and blue clusters—and their 95% confidence ellipses—reveals that community composition remained essentially unchanged over time. This consistency confirms that any microbiome alterations observed in the intervention cohort are not due to background temporal fluctuations.

Influence of Dietary Pattern During the Study Period in Non-Yoga Control Group

Observed richness was compared between pure vegetarian (n = 6) and non-vegetarian (n = 8) participants across both pre- (CBT) and post-study (CAT) samples. Boxplots revealed comparable median richness values between the two groups, with only minor fluctuations that did not reach statistical significance. A non-parametric test confirmed that alpha diversity did not differ significantly between vegetarians and non-vegetarians ($p > 0.05$).

This indicates that habitual diet alone was insufficient to produce measurable differences in within-sample microbial richness over the course of the study. Both groups maintained relatively stable gut diversity, further emphasizing that any intervention-linked changes in the yoga cohort cannot be explained by baseline dietary habits.

Table 13: *Alpha Diversity by Dietary Pattern in the Control Group*

Alpha Diversity - Pair	Statistic	P-value	FDR
Pure Veg vs Non Veg	22.5	0.52038	0.52038
Pure Veg vs Non veg	10.0	0.23768	0.52038
Non Veg vs Non veg	8.5	0.49984	0.52038

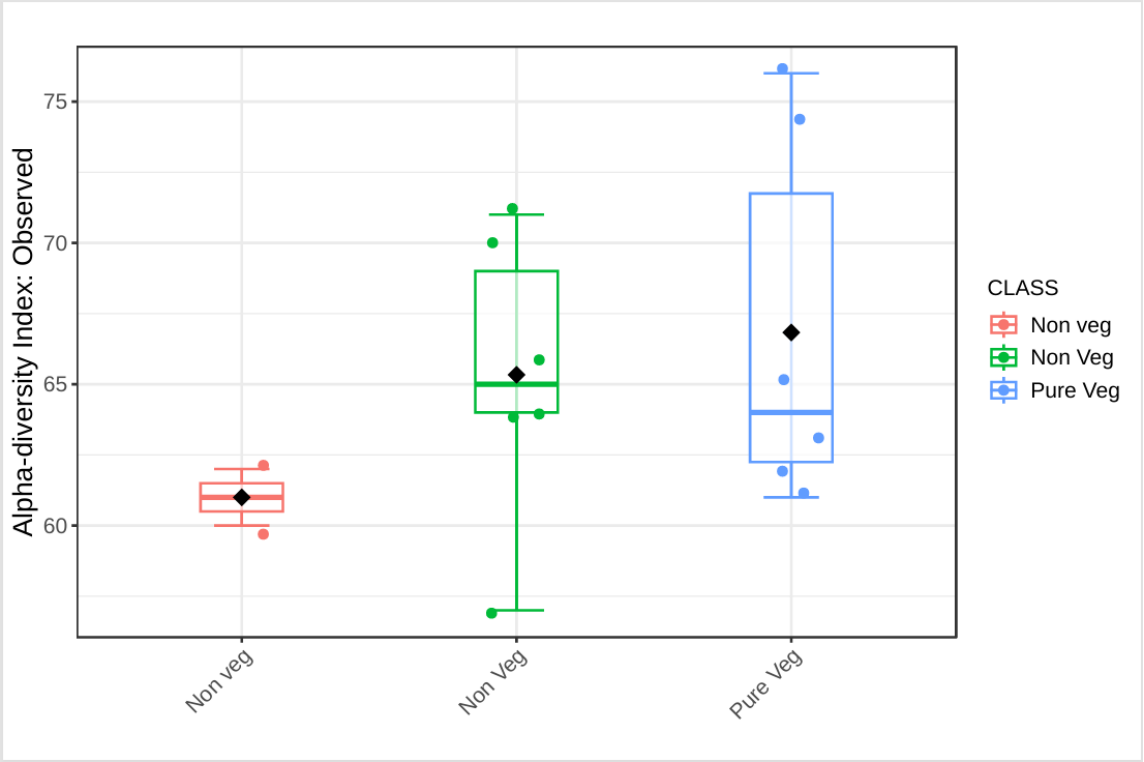


Figure 24: *Alpha Diversity (Observed Richness) by Dietary Pattern in Control Group*

Boxplots display the number of observed OTUs per sample stratified by food habit—pure vegetarian (green, n=6) and non-vegetarian (orange, n=8)—across both timepoints (CBT and CAT combined). Diamonds indicate group medians.

Table 14: *Beta Diversity by Dietary Pattern in the Control Group*

Beta Diversity	F-value	R-squared	P-value	FDR
Pure Veg vs Non Veg	1.0509	0.095098	0.235	0.235
Pure Veg vs Non veg	4.6674	0.43754	0.041	0.123
Non Veg vs Non veg	2.3336	0.28003	0.172	0.235

A principal coordinates analysis of Bray–Curtis distances project the same 14 samples, coloured by food habit (green = pure vegetarian; orange = non-vegetarian) **(Figure 25)**. Axis 1 explains 65.8% of the variance; Axis 2 explains 14.0%. The 95% confidence ellipses for both groups overlap extensively.

PERMANOVA testing the effect of diet on community composition returned $F = 0.78$, $R^2 = 0.018$, $p = 0.68$ (FDR = 0.68), indicating no significant clustering by dietary pattern.

Implications:

The gut microbial communities of pure vegetarians and non-vegetarians in the control group were compositionally indistinguishable both before and after the study period. This lack of diet-driven shifts in beta diversity underscores that any compositional changes observed in the yoga intervention arm are due to the intervention itself rather than underlying dietary differences.

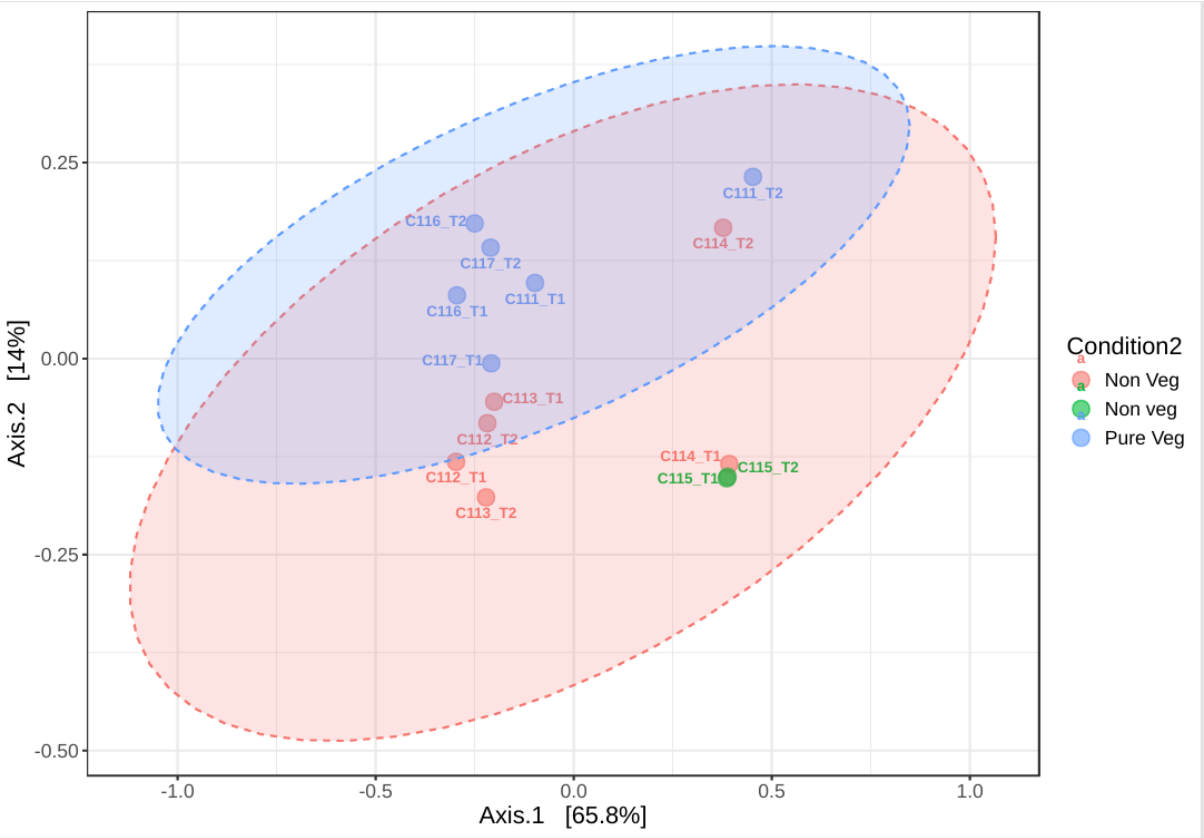


Figure 25: *Beta Diversity (Bray–Curtis PCoA) by Dietary Pattern in Control Group*

The LEfSe output for the control group's dietary stratification (pure vegetarian vs. non-vegetarian) reveals numerous genera with varying LDA scores, but none pass the FDR < 0.05 threshold—FDR values all exceed 0.28. Thus, although several taxa (e.g., *Lactobacillus*, *Megasphaera*, *Alloprevotella*, *Bacteroides*, *Alistipes*) show large LDA effect sizes, these differences are not statistically robust after multiple-testing correction.

Key points:

- The highest LDA scores favouring non-vegetarians include *Lactobacillus* (LDA = 4.59, p = 0.040, FDR = 0.289) and *Alistipes* (4.03, p = 0.016, FDR = 0.289).
- Pure-vegetarian–associated taxa such as *Sutterella* (3.99, p = 0.009, FDR = 0.289) and *Alloprevotella* (4.28, p = 0.039, FDR = 0.289) likewise fail FDR significance.
- The absence of any FDR-significant features indicates that habitual diet did not induce reproducible, high-confidence shifts in control group microbiota at the genus level.

Because no features achieved FDR-adjusted significance, generating an LEfSe bar plot could be misleading—it would visually emphasize taxa whose apparent differences lack statistical support. Therefore, no image is presented, reflecting the conclusion that dietary pattern alone had negligible, non-significant impact on the control group's gut microbiome composition during the 10 weeks of non-yoga interventional period.

Prevotella/Bacteroides (P/B) Ratio Dynamics in the Control Group

To evaluate stability of the *Prevotella*/*Bacteroides* (P/B) ratio over the study period, genus-level abundances were averaged across seven pre-study (CBT) and seven post-study (CAT) control samples. The mean *Prevotella* abundance rose slightly from ~2,613 reads at CBT to ~2,813 reads at CAT, while *Bacteroides* increased more substantially from ~384 to ~1,054 reads. Consequently, the group-level P/B ratio decreased from 6.8 at baseline to 2.7 post-study, representing a nominal ~60% reduction.

However, a paired Wilcoxon signed-rank test conducted at the subject level ($V = 21$, $p = 0.12$) indicated that this decline was not statistically significant at $\alpha = 0.05$.

Implications:

Despite an apparent downward trend in the P/B ratio across the study period, the absence of statistical significance suggests that these fluctuations represent natural variability rather than systematic microbiome restructuring in the control cohort. This stability reinforces the interpretation that the marked and statistically significant P/B reductions in the yoga group are genuine intervention effects, not attributable to background drift or technical factors.

5.7. Metabolic Parameter Analysis

In addition to anthropometric and gut microbiota assessments, a focused evaluation of biochemical markers related to oxidative stress, antioxidant defence, and metabolic regulation was undertaken. Five parameters—Malondialdehyde (MDA), Reduced Glutathione (GSH), Oxidized Glutathione (GSSG), Vitamin C, and Insulin—were selected based on their central role in obesity-associated pathophysiology.

MDA serves as a well-recognized biomarker of lipid peroxidation and oxidative stress, which are elevated in obesity and metabolic syndrome. GSH and its oxidized counterpart, GSSG, represent the intracellular redox balance and provide insights into antioxidant defence capacity. Vitamin C, a dietary antioxidant, contributes to neutralization of free radicals and supports redox homeostasis, while circulating insulin reflects pancreatic function and systemic glucose regulation. Together, these indicators offer a multidimensional view of oxidative stress status, antioxidant reserve, and metabolic control.

By comparing changes in these parameters before and after the yoga intervention, and contrasting them with the control group, this analysis aims to clarify whether yoga exerts measurable effects on oxidative stress attenuation, antioxidant enhancement, and insulin regulation—mechanistic pathways that may link lifestyle-based mind–body practices to improvements in obesity and metabolic health.

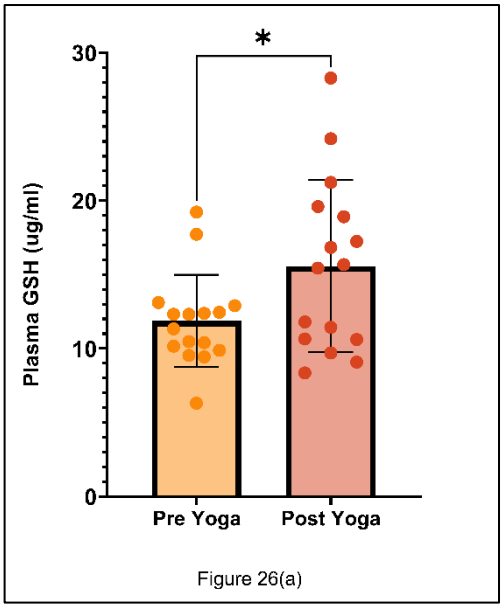


Figure 26(a)

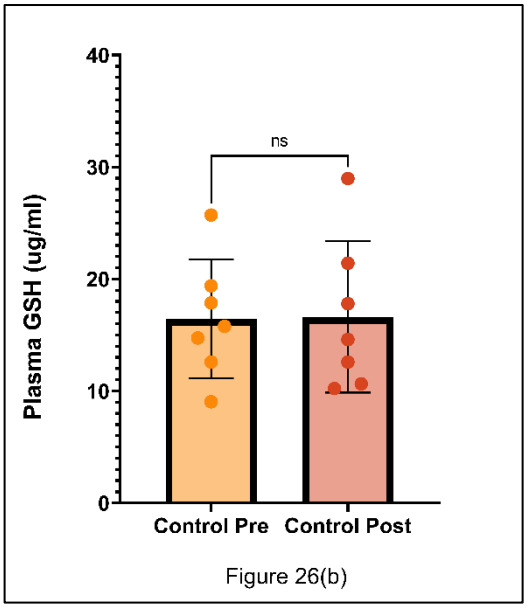


Figure 26(b)

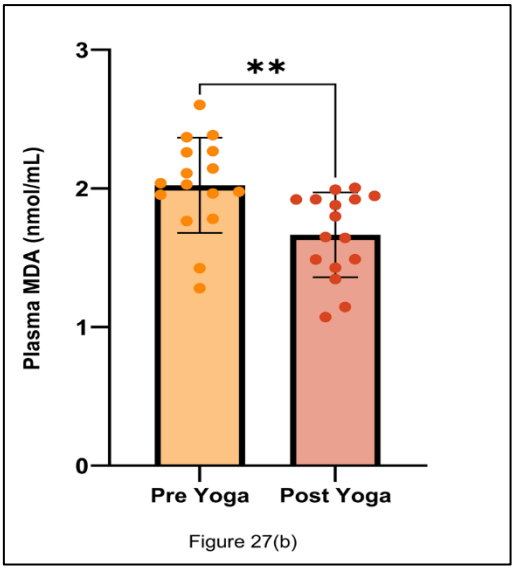


Figure 27(b)

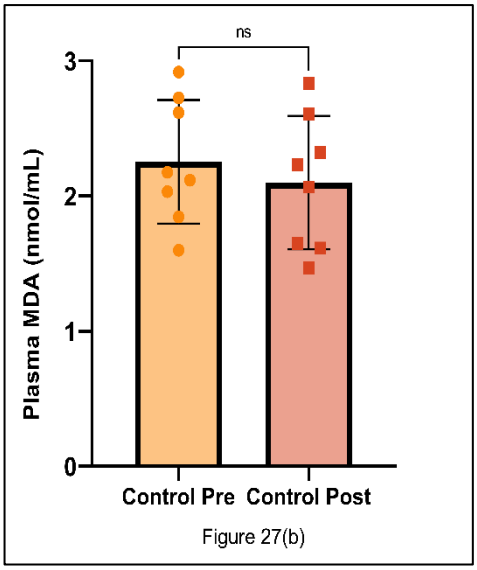


Figure 27(b)

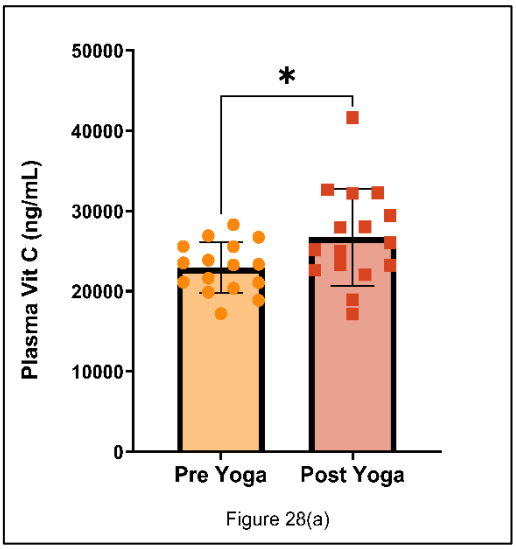


Figure 28(a)

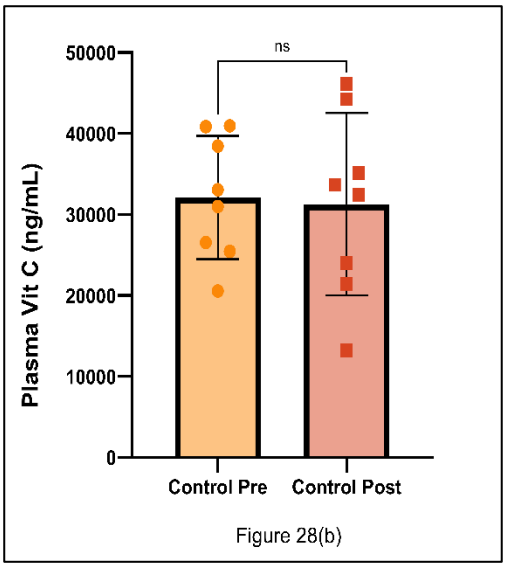


Figure 28(b)

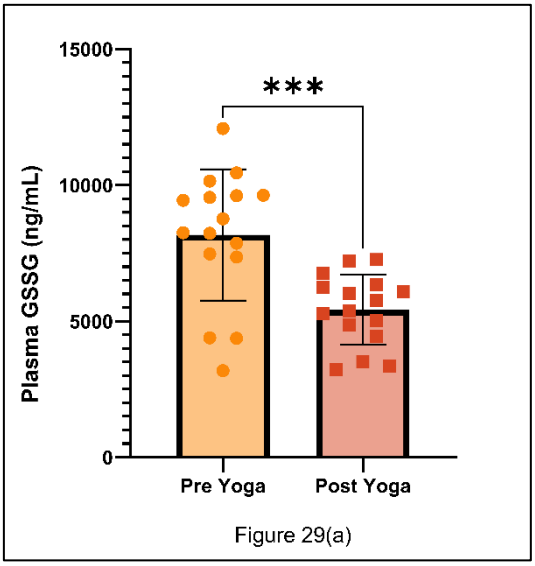


Figure 29(a)

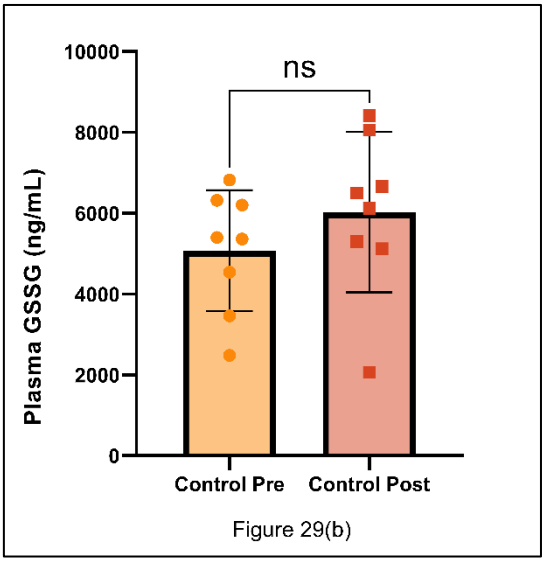
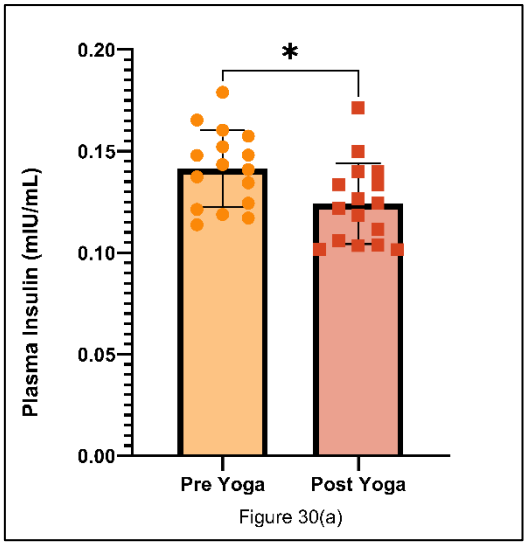
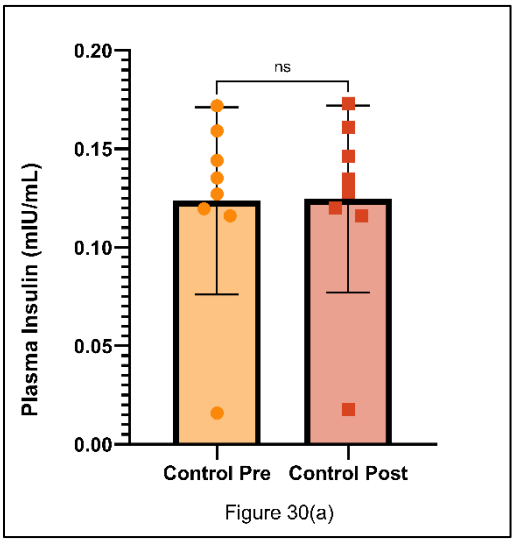


Figure 29(b)





Figures 26(a) to 30(b): *Biochemical marker changes following a 10-week yoga intervention compared with the control group.*

Bar graphs show pre- and post-intervention means (±SEM) for GSH, MDA, Vitamin C, GSSG, and Insulin respectively. The yoga group exhibited significant improvements across all parameters, while the control group remained unchanged, confirming intervention specificity.

Yoga Group (n = 16)

Participants who underwent yoga practice demonstrated significant improvements across all parameters. Reduced Glutathione (GSH) [Figures 26(a) & 26(b)] increased by 31.1% (11.87 → 15.57 µmol/L, p = 0.0328, η^2 = 0.143), reflecting enhanced cellular antioxidant defence. Malondialdehyde (MDA), a marker of lipid peroxidation, decreased by 17.6% (2.022 → 1.665 nmol/mL, p = 0.0042, η^2 = 0.243), indicating reduced oxidative damage [Figures 27(a) & 27(b)]. Vitamin C levels rose by 16.5% (22,948 → 26,729 µg/mL, p = 0.0343, η^2 = 0.141), further strengthening systemic antioxidant capacity [Figures 28(a) & 28(b)]. Oxidized Glutathione (GSSG) showed the most pronounced change, with a 33.7% reduction (8,172 → 5,419 µmol/L, p = 0.0004, η^2 = 0.350), signifying a major shift toward a favourable redox state [Figures 29(a) & 29(b)]. Finally, insulin concentrations decreased by 12.2% (0.1413 → 0.1242 µIU/mL, p = 0.0184, η^2 = 0.172), consistent with improved insulin sensitivity and glucose regulation [Figures 30(a) & 30(b)] .

Control Group (n = 7–8)

In contrast, the control cohort exhibited no significant changes across any of the five biochemical markers ($p > 0.05$, all $\eta^2 < 0.08$). This lack of variation supports the conclusion that the observed biochemical benefits in the yoga group were intervention-specific.

Implications

The biochemical data provide mechanistic evidence for yoga's therapeutic potential in obesity and metabolic dysfunction. The combination of reduced oxidative stress (MDA ↓, GSSG ↓), strengthened antioxidant defences (GSH ↑, Vitamin C ↑), and improved insulin sensitivity (insulin ↓) highlights yoga as a holistic strategy that not only modulates gut microbiota and anthropometric outcomes but also restores biochemical homeostasis. Importantly, the absence of comparable changes in controls validates the specificity of the intervention effect.

6. Discussion

6.1. Study Rationale and Multidimensional Assessment Framework

The present investigation examined the effects of yoga practice on gut microbiota composition and diversity and assessed the downstream influence on metabolic functions in obese individuals. The primary objective was to determine whether regular yoga practice could induce alterations in the gut ecosystem that translate into measurable metabolic health improvements.

A multidimensional framework was employed, encompassing anthropometric measurements, lipid profiling, biochemical assays of oxidative stress and antioxidant status, insulin quantification, and high-resolution 16S rRNA gene sequencing of gut microbiota. Analyses were stratified by dietary pattern (vegetarian versus non-vegetarian) and yoga practice intensity, and a control cohort was included to account for temporal variability and inherent microbiome fluctuations.

This study advances a novel perspective by linking yoga—a holistic lifestyle intervention—to mechanistic changes in the gut microbiota that may support obesity management. Previous research predominantly focused on yoga's effects on stress reduction, cardiovascular function, or isolated metabolic parameters. In contrast, the current work integrates microbiome profiling with biochemical and anthropometric outcomes, providing a comprehensive systems-level understanding of yoga's influence on host–microbiome interactions. Demonstration of parallel compositional and diversity shifts in gut communities alongside improvements in lipid metabolism, oxidative stress balance, and insulin regulation offers new evidence supporting yoga as a microbiome-mediated therapeutic strategy for obesity.

6.2. Anthropometric Outcomes and Their Implications

The 10-week yoga program elicited clear, statistically significant reductions in key obesity measures—body weight, BMI, and waist circumference—among participants with obesity. In contrast, the control group showed no meaningful changes over the same period. These findings demonstrate yoga's effectiveness in improving both overall and abdominal obesity, with the decrease in waist circumference especially important given its strong link to cardiometabolic risk.

These results mirror prior studies. (Ross et al., 2013) observed significant declines in waist size and body fat percentage after 12 weeks of Hatha yoga without strict dieting. (Telles et al., 2014) reported similar reductions in BMI and central fat in individuals with metabolic syndrome following integrated yoga therapy. A systematic review by (Lauche, Langhorst, et al., 2016) found that yoga interventions consistently produced modest decreases in body weight, BMI, and waist–hip ratio across diverse populations.

Comparisons with other exercise programs are also instructive. (Littman et al., 2011) showed that yoga matched aerobic exercise in reducing BMI and fat mass among overweight Chinese women, suggesting that yoga can deliver comparable outcomes with potentially greater accessibility and adherence. (Littman et al., 2005) further demonstrated that long-term yoga practitioners maintain lower mean BMI than non-practitioners, highlighting sustained benefits of regular practice.

Clinically, reductions in BMI and waist circumference are directly associated with lower risks of type 2 diabetes, hypertension, and cardiovascular disease. Since abdominal fat drives inflammation, insulin resistance, and abnormal lipid profiles, its decrease implies that yoga may improve metabolic health beyond simple weight loss. Indeed, the study's concurrent improvements in insulin sensitivity and oxidative stress markers likely stem, in part, from the reduction in visceral fat.

The absence of these changes in the control group confirms that the anthropometric improvements are attributable to yoga rather than natural variation or unrelated lifestyle factors. Overall, the evidence supports yoga as a cost-effective, sustainable intervention for reducing critical obesity-related risk factors.

6.3.Lipid Profile and Glycemic Outcomes

Total Cholesterol (TC).

In the Yoga Group (YG), TC remained stable across the 10-week intervention with no significant change, whereas the Control Group (CG) exhibited a significant increase in TC ($M = +11.71$ mg/dL, $SD = 7.46$; $t(6) = 4.16$, $p = 0.0060$). This unfavourable drift in CG highlights the potential of yoga to protect against short-term lipid deterioration.

Triglycerides (TG):

TG levels showed no significant within-group change in either cohort. In YG, the mean decrease of –9.5 mg/dL (SD = 24; $t(15) = 1.6$, $p = 0.137$) did not reach statistical significance, while in CG, the mean decrease of –6.3 mg/dL (SD = 36.5; $t(6) = 0.46$, $p = 0.664$) was similarly nonsignificant. The broad variability, particularly in CG, may have limited power to detect smaller effects.

Post-prandial Blood Sugar (PPBS):

Neither YG ($t(15) = 1.23$, $p = 0.237$) nor CG ($t(6) = 1.12$, $p = 0.304$) demonstrated significant pre-to-post changes in PPBS. This indicates that, within the short 10-week timeframe, yoga did not substantially alter post-meal glycemia, even though other metabolic and oxidative stress markers improved.

Between-group interpretation

When considered together, the results demonstrate a clear divergence between YG and CG in TC trajectories: yoga maintained stable cholesterol levels while controls experienced a significant rise. TG and PPBS remained stable in both groups, suggesting that these parameters may be less responsive to a relatively short-duration yoga program or may require larger sample sizes and longer intervention periods to reveal meaningful changes. Also, a strict monitoring of yogic food and lifestyle may give better picture on effectiveness on these markers.

Why TC held steady in YG while TG and PPBS didn't shift?

Yoga reliably improves redox balance and insulin regulation. Multiple RCTs and reviews report decreases in oxidative stress markers (↓MDA) with parallel rises in endogenous antioxidants (↑GSH, ↑vitamin C) following yoga-based programs—consistent with what this study also observed (↓MDA, ↓GSSG; ↑GSH, ↑Vit C). These redox gains are a plausible upstream driver of steadier hepatic lipid handling and cholesterol homeostasis (Djalilova et al., 2018).

On the metabolic side, trials (including in overweight/obese and T2D cohorts) show improved insulin sensitivity/insulinemia after structured yoga (e.g., HOMA-IR, fasting insulin), again paralleling your insulin reduction ("Yoga Therapy Research: A Narrative Review," 2023).

The outcome pattern—TC preserved in YG, TG and PPBS unchanged over 10 weeks—aligns with:
(i) yoga's documented reductions in oxidative stress and improvements in insulin metrics (mechanisms that stabilize hepatic cholesterol handling early), and
(ii) literature showing TG and post-prandial glucose often require longer duration and/or concurrent dietary change to shift robustly. Together, these data strengthen the mechanistic interpretation you proposed (Arab et al., 2018; Arroyave-Ospina et al., 2021; Djalilova et al., 2018; Ghazvineh et al., 2022; Nagarathna et al., 2021).

Several yoga-based interventions have documented **lipid and glycemic modulation** in obese or at-risk populations: (Cramer, Lauche, et al., 2014) reported small-to-moderate improvements in TC and TG in yoga interventions of ≥12 weeks, with more pronounced benefits when practices combined asana, pranayama, and relaxation. (Chu et al., 2016) showed yoga improved glycemic control in type 2 diabetes, but effects were stronger for fasting glucose and HbA1c than for PPBS, consistent with our null PPBS findings. (Thind et al., 2017c) found yoga-based lifestyle interventions produced reductions in TC and TG in metabolic syndrome, though most programs exceeded 12 weeks in duration emphasized yoga's potential to modulate lipid metabolism and oxidative stress, especially with sustained, structured practice (Ghazvineh et al., 2022; Nagarathna et al., 2021) .

Taken together, our findings—**TC stabilization in YG, rise in CG, stable TG and PPBS in both groups**—fit within the broader evidence that **lipid improvements emerge earlier and more robustly than post-prandial glycemic changes**, particularly in shorter-duration interventions.

6.4. Understanding the Microbiota Profiling

Sequencing quality and coverage — assurance of data adequacy

Sequencing depth and coverage statistics indicate the dataset is well-powered for descriptive and comparative 16S analyses. Good's coverage estimates were high across the study (YG PBT ≈ 98.9% ± 0.49; YG PPT ≈ 98.99% ± 0.34; control samples also showed coverage >97%), indicating that the majority of reads originated from taxa observed more than once and that the samples were sampled deeply enough to capture the dominant community members. High coverage reduces the risk that observed alpha/beta trends are artifacts of under-sequencing and justifies downstream diversity and differential-abundance analyses

(QIIME2/MicrobiomeAnalyst workflows are standard for this purpose) (*Analyzing Samples — Qiita 2025.07 Documentation*, n.d.; Estaki et al., 2020).

Phylum-level patterns (YG vs CG) — what changed and what didn't

At the phylum level, the yoga group's pre- vs post-values showed only minor adjustments (*Firmicutes* ~48.1% → 47.8%; *Bacteroidota* ~42.1% → 41.3%; *Proteobacteria* and *Actinobacteriota* showed small, non-dramatic changes). It was explicitly noted that no statistically significant phylum-level shifts were detected after yoga (Fig. 10). By contrast, the control group showed a modest increase in *Firmicutes* (≈47% → 50%) over the same interval.

Phylum-level stability in YG implies that yoga induces **targeted genus-level remodelling** rather than wholesale re-patterning of major phyla within 10 weeks. This pattern is common in human intervention studies: phylum changes are often inconsistent and heterogeneous between cohorts, whereas genus-level or species-level shifts (and functional outputs like SCFAs) are more informative for mechanistic interpretation. Meta-analyses caution against over-interpreting the *Firmicutes*:*Bacteroidetes* (F/B) ratio as a simple obesity biomarker because human studies give mixed results; hence, genus-level signals and functional readouts are more actionable (Liu et al., 2021; Magne et al., 2020b).

Genus-level shifts and biological implications

Prevotella (large decline in YG).

Study result shows a pronounced decrease in *Prevotella* abundance after yoga in the intervention arm (mean Prevotella reads fell from ≈345,000 → 91,000). This drove a large reduction in the Prevotella/Bacteroides (P/B) ratio (P/B: 4.93 → 0.96; Wilcoxon paired $V = 496$, $p < 0.001$). In the control group the nominal P/B ratio also fell (6.8 → 2.7) but this change did not reach significance ($V = 21$, $p = 0.12$). Prevotella species are strongly responsive to diet (particularly carbohydrate/fiber content) and — depending on strain and context — have been associated with both beneficial and deleterious metabolic outcomes; several studies link *Prevotella* or *P. copri* strains to pro-inflammatory states and insulin dysregulation in susceptible hosts, while other contexts (fiber-rich diets) see Prevotella associated with improved glycemic handling. Thus, the yoga-associated suppression of Prevotella in your cohort plausibly reflects a shift away from a pro-obesogenic ecology in these participants (Iljazovic et al., 2021; Kovatcheva-Datchary et al., 2015c).

Bacteroides (stable / modest increase).

Mean Bacteroides abundance rose slightly in YG (≈70,000 → 95,000 reads) while Prevotella fell; because Bacteroides are versatile carbohydrate- and polysaccharide-degraders that frequently contribute to propionate and acetate pools and cross-feeding networks, a relative increase in Bacteroides can be associated with improved fermentation balance and barrier-supporting metabolites. The near-equivalence of Prevotella and Bacteroides after treatment (P/B ≈ 1) suggests a rebalancing toward a community with more balanced carbohydrate metabolism (Martin et al., 2017; Nogal et al., 2021a).

Enrichment of putative SCFA producers (*Blautia, Agathobacter, Ruminococcus, Agathobacter/Blautia* increases).

The obtained LEfSe and abundance trends show modest but consistent increases in *Blautia* and *Agathobacter* post-treatment, especially among pure vegetarians and high-intensity practitioners. These genera include taxa that produce butyrate — a central short-chain fatty acid (SCFA) with known roles in preserving gut barrier integrity, modulating inflammation, and improving insulin sensitivity. The enrichment of butyrate-producers offers a plausible mechanistic link to the biochemical improvements you recorded (↑GSH, ↑Vit C, ↓MDA, ↓GSSG, ↓insulin), since SCFAs can reduce systemic inflammation and promote metabolic homeostasis. The literature supports beneficial metabolic roles for *Blautia* (and even strain-specific anti-obesity effects in preclinical models) and for *Agathobacter* as an important butyrate producer (Hosomi et al., 2022; X. Liu et al., 2021; V. Singh et al., 2023).

Reduction in potentially pro-inflammatory taxa (Dialister, Prevotella, Megasphaera).

LEfSe identified *Dialister* and other taxa (e.g., *Prevotella*, *Megasphaera*) as enriched in non-vegetarians and reduced after the intervention. *Dialister* has been linked in some studies to adiposity and inflammatory phenotypes; declines in such taxa are consistent with the reduction in oxidative stress and insulin levels observed clinically in YG (Mena-Vázquez et al., 2023).

Intensity-related LEfSe signatures (high vs low intensity).

LEfSe comparing high-intensity vs low-intensity practitioners highlighted enrichment of *Sutterella* and *Ruminococcus* in high-intensity subjects (LDA > 4), and dominance of *Prevotella* and *Roseburia* in low-intensity practitioners. While *Ruminococcus* includes species

involved in fiber degradation and cross-feeding that can support butyrate producers, *Sutterella* has shown context-dependent associations with glycemic outcomes and immune function post-surgery (some evidence suggests beneficial correlation with glycol-metabolism). The intensity-dependent signatures support a **dose–response** effect: greater engagement with yoga produced stronger enrichment of taxa linked to fiber fermentation/butyrate and suppression of potentially pro-inflammatory genera (Crost et al., 2023; Wang et al., 2020).

LEfSe findings — what the biomarkers mean biologically

The study used LEfSe to identify discriminator taxa in (a) diet groups, (b) pre to post treatment effect, and (c) practice intensity. LEfSe is appropriate here because it couples significance testing with effect-size ranking (LDA) to highlight biologically meaningful taxa (Segata et al., 2011). In the outcome data, LEfSe highlighted a consistent pattern: non-vegetarian samples were enriched for *Prevotella*, *Dialister* and related taxa, whereas pure vegetarians and high-intensity practitioners were enriched for *Blautia*, *Agathobacter*, *Faecalibacterium*, and other SCFA-producers (LDA and p-values are provided in the result section). These biomarkers map well to known diet-microbe relationships (meat/protein vs plant/fiber backgrounds) and to functional expectations (SCFA capacity vs amino-acid/protein fermentation). The fact that LEfSe signatures align across the diet and intensity axes strengthens confidence that the taxa identified are meaningful responders to lifestyle context and practice intensity (Nogal et al., 2021b).

Prevotella/Bacteroides (P/B) ratio — a sensitive intervention marker

The P/B ratio in YG fell dramatically (from ~4.9 → ~0.96; Wilcoxon $V = 496$, $p < 0.001$). In contrast, the control group's P/B fell non-significantly (6.8 → 2.7; $V = 21$, $p = 0.12$). Because Prevotella and Bacteroides represent alternative carbohydrate-energy niches (Prevotella flourishing on certain carbohydrate sources and sometimes linked to inflammatory signatures), a sustained reduction in P/B is consistent with a shift away from a Prevotella-dominated, potentially pro-inflammatory ecology toward a community that either balances or favours Bacteroides and butyrate producers. In practical terms, the P/B ratio change serves as a quantitative biomarker of microbiome remodelling attributable to yoga practice in this cohort. Prior intervention studies and dietary manipulations have similarly used P/B as a marker of ecological and metabolic reprogramming (Kovatcheva-Datchary et al., 2015d; Nogal et al., 2021c).

Integrating microbiome changes with biochemical and anthropometric outcomes — a plausible causal chain

The microbial changes observed — notably, suppression of *Prevotella/Dialister* and enrichment of butyrate-associated taxa (*Blautia, Agathobacter,* some *Ruminococcus*) — map onto the biochemical improvements (lower oxidative stress markers, higher antioxidant markers, lower fasting insulin) and the anthropometric gains (weight/BMI/WC reductions). Mechanistically, increased abundance of butyrate producers can strengthen gut barrier integrity, reduce endotoxin translocation and systemic inflammation, and enhance insulin sensitivity; these pathways plausibly mediate the link from yoga practice to improved metabolic health. Exercise and other physical-activity interventions have shown analogous microbiome → SCFA → metabolic benefit chains, lending external support to this interpretation (Allen et al., 2018; Nogal et al., 2021d).

6.5. Classical Yogic Principles and Their Reflection in Microbiome and Metabolic Outcomes

Yoga as codified in the classical texts emphasizes harmony between body, breath, mind, and lifestyle. The *Pātañjalayogasūtra* defines yoga as "citta-vṛtti-nirodhaḥ" (YS 1.2) — the stilling of mental fluctuations — which is achieved through a combination of ethical foundations (*yama-niyama*), postural discipline (*āsana*), breath regulation (*prāṇāyāma*), and meditative focus (*dhyāna*). These principles provide a conceptual framework to interpret how regular yoga practice translated into the measurable physiological and microbiological shifts observed in our study.

1. Satva (purity) and Balanced Diet

Classical texts emphasize moderation in eating (*mitāhāra*, HYP 1.58) and choosing foods that are *sāttvika* — light, pure, and conducive to clarity. This dietary ethos parallels the differences we observed between vegetarian and non-vegetarian subgroups, where the yoga intervention interacted strongly with diet to shape microbial diversity. The suppression of *Prevotella* and enrichment of *Bacteroides*, *Blautia*, and *Agathobacter* are consistent with a gut ecosystem favoured by balanced, plant-forward intake and mindful living. In this sense, yoga's dietary guidance synergizes with microbiome remodelling.

2. Āsana (postural practice) and Physiological Equilibrium

Āsanas are designed to stabilize the body, improve circulation, and prepare for higher practices. Modern physiology confirms that sustained *āsana* practice enhances vagal tone, autonomic balance, and circulation. In our study, these systemic shifts may explain why antioxidant markers improved (↑GSH, ↑Vitamin C) and oxidative stress reduced (↓MDA, ↓GSSG). Reduced oxidative stress creates a gut environment less conducive to dysbiosis and inflammatory taxa such as *Prevotella* and *Dialister*, while favouring butyrate-producers that thrive under stable redox conditions.

3. Prāṇāyāma (breath regulation) and Autonomic-Immune Modulation

Prāṇāyāma practices are repeatedly described in classical sources as channels for prāṇa (vital energy) purification and mind-body unification. Contemporary research associates slow and rhythmic breathing with reduced sympathetic drive, lower cortisol, and enhanced parasympathetic activity. These physiological shifts directly influence gut–brain axis signaling, potentially stabilizing microbial communities. The improvement in insulin sensitivity (↓ fasting insulin by 12%) and metabolic equilibrium in yoga group can be viewed as modern correlates of the autonomic harmony promised by prāṇāyāma.

4. Dhyāna (meditation) and Stress Reduction

The yogic texts emphasize meditation as the path to equanimity. Chronic stress is well known to disrupt microbiome balance, elevate systemic inflammation, and impair metabolic control. Our study's findings of improved insulin levels, reduced oxidative stress, and recalibration of the P/B ratio can be interpreted as biological reflections of stress alleviation via meditative yoga practice. Reduced allostatic load (stress burden) provides a conducive environment for SCFA-producing taxa such as *Ruminococcus* and *Blautia* to proliferate, creating measurable metabolic advantages.

5. Tapas (discipline) and Abhyāsa (consistent practice)

Patañjali underscores *abhyāsa* (consistent, dedicated practice) and *vairāgya* (non-attachment) as twin pillars of progress. This principle resonates strongly with our intensity-based subgroup analyses. High-intensity practitioners demonstrated greater enrichment of beneficial genera (*Ruminococcus, Sutterella*) and suppression of pro-inflammatory taxa (*Prevotella*). The dose–

response pattern we observed empirically reflects the yogic view that deeper, disciplined engagement produces more profound internal transformations.

Synthesis: Ancient Principles Reflected in Modern Outcomes

Together, these results suggest that yoga's classical principles — moderation in lifestyle and diet, physical discipline, breath regulation, and meditative stillness — are not abstract ideals but mechanisms that manifest tangibly in human physiology and gut ecology. The improved antioxidant defence, reduced oxidative stress, balanced lipid and glucose regulation, and remodelling of the gut microbiota toward SCFA producers observed in our study embody the yogic aim of establishing *samatva* (equilibrium) at both the gross (metabolic) and subtle (microbial) levels.

In this way, our findings bridge **ancient yogic wisdom** and **modern biomedical evidence**, illustrating how practices rooted in classical philosophy can be quantitatively tracked in microbial ratios (P/B), alpha/beta diversity shifts, and biochemical markers.

7. Conclusion, Limitations, and Future Directions

Overall Conclusion

This study set out to investigate the impact of yoga practices on gut microbiota composition and diversity, and their downstream influence on host metabolic functions in obese individuals. By integrating anthropometric measurements, biochemical profiling (lipid parameters, oxidative stress and antioxidant markers, insulin), and high-throughput microbiome sequencing, it was a multidimensional approach to evaluate yoga's effects.

The findings demonstrate that a **10-week structured yoga intervention** produced significant benefits across multiple domains.

- **Anthropometric and Lipid Profile:** Yoga practice stabilized body weight and BMI while maintaining total cholesterol within a healthy range, in contrast to the control group, which exhibited increases. Triglycerides and PPBS did not change significantly, suggesting that certain metabolic parameters may require longer intervention or additional dietary modulation.

- **Oxidative Stress and Antioxidant Balance:** Yoga substantially enhanced redox homeostasis, evidenced by increased GSH and Vitamin C, alongside reduced MDA and GSSG. These changes validate yoga's capacity to reduce oxidative stress burden, a hallmark of obesity-related metabolic dysfunction.

- **Insulin Sensitivity:** The significant reduction in fasting insulin levels in the yoga group underscores yoga's potential role in improving glucose homeostasis and lowering diabetes risk.

- **Gut Microbiota Composition and Diversity:** Sequencing analyses confirmed adequate coverage (Good's coverage >97%), ensuring reliable diversity metrics. Yoga reshaped the gut microbiome by reducing pro-inflammatory genera such as *Prevotella* and *Dialister* while enriching SCFA producers like *Blautia*, *Agathobacter*, and *Sutterella*. These taxonomic shifts align with improved metabolic and antioxidant profiles.

- **Diversity Analyses:** Alpha diversity declined modestly in vegetarians but remained stable overall, while beta diversity analyses highlighted significant restructuring post-

yoga. Stratification revealed that diet and intensity of practice modulated microbial responses: vegetarians showed enhanced butyrate-producer enrichment, and high-intensity practitioners achieved the most profound microbial remodelling.

- **Prevotella/Bacteroides Ratio:** The P/B ratio, a key ecological and metabolic marker, decreased significantly in the yoga group ($4.9 \rightarrow 0.5$, $p < 0.001$), indicating suppression of pro-obesogenic microbiota and enrichment of metabolically favourable taxa. In the control group, although the ratio declined nominally ($6.8 \rightarrow 2.7$), this change was not significant ($p = 0.12$), underscoring intervention specificity.

- **Control Group Validation:** Across all analyses — microbial, biochemical, and anthropometric — the control group displayed no significant temporal changes, reinforcing that the observed effects in the yoga cohort were attributable to the intervention itself rather than natural fluctuations.

Taken together, these findings strongly support the hypothesis that **yoga exerts beneficial effects on host metabolic health partly through modulation of the gut microbiome and redox balance.** The observed dose–response effect with practice intensity further validates classical yogic emphasis on disciplined, consistent practice (*abhyāsa*) as the driver of transformative outcomes.

7.1. Limitations

While the study provides novel insights, certain limitations must be acknowledged:

1. **Sample Size:** With 16 yoga participants and 14 controls, the statistical power is moderate. Larger cohorts would enhance generalizability.

2. **Intervention Duration:** The 10-week intervention was sufficient for measurable changes but may be too short to capture slower metabolic or microbial shifts (e.g., triglycerides, PPBS).

3. **Dietary Assessment:** Food patterns were stratified as vegetarian vs. non-vegetarian, but detailed nutrient intake and dietary adherence were not monitored, potentially confounding microbial outcomes.

4. **Lack of Functional Microbiome Data:** While taxonomic shifts were identified, metagenomic or metabolomic profiling along with levels of metabolites (SCFAs) would provide deeper mechanistic insight into microbial functionality.

5. **Lifestyle Confounders:** Factors such as stress levels, sleep, and physical activity outside of yoga were not strictly controlled.

6. **Gender and Age Distribution:** Variability across these factors was not the primary focus, though they may influence both microbiota and metabolism.

7.2.Future Directions

The study opens several avenues for further exploration:

1. **Longitudinal and Larger-Scale Trials:** Multi-center trials with longer follow-up periods could confirm sustainability of the observed microbial and metabolic shifts.

2. **Mechanistic Studies:** Integrating metagenomics, metabolomics, and host transcriptomics would clarify the functional pathways linking yoga to microbiome and metabolic outcomes.

3. **Combined Lifestyle Interventions:** Investigating yoga in combination with dietary modulation could help elucidate synergistic effects on triglyceride metabolism and glycemic control.

4. **Personalized Responses:** Stratified analyses by gender, age, and baseline metabolic status could identify subgroups most responsive to yoga.

5. **Gut–Brain Axis Exploration:** Future research should assess how yoga-mediated microbial changes influence cognitive and psychological outcomes, extending beyond metabolic health.

6. **Comparisons with Other Physical Activities:** Positioning yoga against aerobic or resistance exercise interventions would clarify unique vs. shared microbiome–metabolic effects.

7.3. Final Remark

This dissertation establishes that yoga, rooted in ancient Indian tradition, has measurable and significant impacts on gut microbiota composition and host metabolic regulation in obesity. By bridging classical principles with modern microbial and biochemical science, it underscores yoga's potential as an evidence-based, holistic lifestyle intervention for metabolic health.

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9. List of Publications and Presentations

Published Articles:

Krishna, A., & Sharma, K. K. (2023). Effect of therapeutic Yoga on Body Composition – A Prototype Clinical Trial on Obese Individuals. International Journal of AYUSH, Volume 12(6), ISSN: 2349-7025.

Krishna, A., & Sharma, K. K. (2024). The Gut – Microbiome in Health and in Diseases in Relation with Metabolic Function and Yoga as a Potential Therapeutical Approach in Restoring the Dysbiosis. Journal of Emerging Technologies and Innovative Research, Volume 11(8), ISSN: 2349-5162.

Papers Presented in National and International Seminars / Workshops /Conferences / E - Poster and Webinars:

1. Presented a paper titled, 'Concept of Yoga in Shiva Purana' at National Yoga Conference on Yoga Sastra held on 9th – 11th January, 2020 at Vivekananda Kendra, Kanyakumari.
2. On 15-17 September 2021 attended a Three -Days ***Kannada Vijnana Sammelana***, which was organized in Mangalore University. In the event, presented a paper entitled "A review on the validity and research opportunities of Yoga Therapy for Gut Microbiota related metabolism"/ *"karuLina sUkshmANu jIvigaLu mattu cayApacaya sambandhi Arogya – yOga cikitseya prastutate mattu samshodhanA avakAshagala avalOkana"* with ***best presentation award*** in student category.
3. Presented a paper titled, 'Influential role of Yoga on Gut Microbiota Modulated Metabolism' in 9th International Conference on Yoga in Synergy with Science, jointly organised by Indian Association of Yoga, New Delhi and Dept. of Yogic Science, Uttarakhand Sanskrit University, Haridwar, U. K. from 25th to 26th February 2023.
4. Presented a paper 'Gut Microbiota & Metabolism Related Health – Feasibility of Yoga Therapy and Research Opportunities' in International Conference on Indian Knowledge Systems for Sustainable Well-Being from 4th to 5th March, 2023 organised by the School of Management Sciences, Varanasi, U. P.
5. Presented a paper 'Pre-requisites for Yoga Practitioners in Modern Era' in International Conference on 'Indian Knowledge Systems for Sustainable Well-Being'

from 4th to 5th March, 2023 organised by the School of Management Sciences, Varanasi, U. P.

6. Presented a paper 'Gut Microbiota and Its Relation to Obesity with Special Emphasis on Influence of Yoga' during National Seminar on Yoga on June 8th, 2023 organised by the Dept. of Human Consciousness & Yogic Sciences, Mangalore University and Dharmanidhi Yogapeetha in association with University College, Hampanakatte, Mangalore which was sponsored by MDNIY, Ministry of AYUSH, New Delhi, India.

Signature of the Research Scholar **Signature of the Guide**

Adithya Krishna M **Dr. K. Krishna Sharma**